UNIVERSITY OF YAOUNDE I
UNIVERSITE DE YAOUNDE I

FACULTY OF SCIENCE
FACULTE DES SCIENCES

DEPARTMENT OF PLANT BIOLOGY
DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VEGETALES

LABORATORY OF PHYTOPATHOLOGY
LABORATOIRE DE PHYTOPATHOLOGIE

Characterization and efficacy of *Beauveria bassiana* and *Metarhizium anisopliae*, two candidates entomopathogenic fungi for the development of a biopesticide for microbial control of banana weevil, *Cosmopolites sordidus*

Thesis submitted in fulfillment of the requirement for the award of a Doctorate/Ph.D. degree in Plant biology

OPTION: Biotechnology

PRESENTED BY: MEMBANG Gertrude

REGISTRATION NUMBER: 05R293

(Master of Sciences)
SUPERVISORS:

HANNA Rachid
Principal Scientist
IITA-Cameroon

AMBANG Zachee
Professor
University of Yaoundé I

Year 2021

UNIVERSITE DE YAOUNDE I
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FACULTY OF SCIENCE

DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VEGETALES
DEPARTMENT OF PLANT BIOLOGY

ATTESTATION DE CORRECTION

****

Nous soussignés, membres du Jury de soutenance de la thèse de Doctorat/Ph.D en Biologie des Organismes Végétaux option Biotechnologies Végétales de l'étudiante **MEMBANG Gertrude**, Matrieule **05R293**, soutenue publiquement le 19 Octobre 2021 sur le sujet «**Characterization and efficacy of *Beauvaria bassiana* and *Metarhizium anisopliae*, two candidates entomopathogenic fungi for the development of a biopesticide for microbial control of banana weevil, *Cosmopoliteq sordidus***» attestons que les corrections conformément aux remarques et recommandations du jury lors de la soutenance de la dite thèse de Doctorat/Ph.D ont été effectuées par la candidate.

En foi de quoi, la présente attestation lui est délivrée pour servir et valoir ce que de droit./-

Rapporteur

AMBANG Zachée
Professeur

Membre

BELL Joseph Martin
Professeur

Président

YOUMBI Emmanuel
Professeur

THE UNIVERSITY OF YAOUNDÉ I **Faculty of Science** Division of Programming and Follow-up of Academic Affairs		UNIVERSITE DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques
LIST OF PERMANENT TEACHING STAFF		**LISTE DES ENSEIGNANTS PERMANENTS**

ACADEMIC YEAR 2019/2020
(By Department and by Grade)
DATE UPDATED: 19 February 2020

ADMINISTRATION

DEAN: TCHOUANKEU Jean-Claude, *Associate Professor*
VICE-DEAN / DPSAA : DONGO Etienne, *Professor*
VICE-DEAN / DSSE : AJEAGAH Gideon AGHAINDUM, *Associate Professor*
VICE-DEAN / DRC : ABOSSOLO Monique, *Associate Professor*
Head of Administration and Financial Division (DAF): NDOYE FOE Marie C. F., *Associate Professor*
Head of Division of Education, Research and Academic Affairs (DAASR): MBAZE MEVA'A Luc Léonard, *Professor*

1- DEPARTMENT OF BIOCHEMISTRY (BCH) (38)			
N°	SURNAME AND GIVEN NAMES	GRADE	OBSERVATIONS
1	BIGOGA DIAGA Jude	Professor	In service
2	FEKAM BOYOM Fabrice	Professor	In service
3	FOKOU Elie	Professor	In service
4	KANSCI Germain	Professor	*Head of Department*
5	MBACHAM FON Wilfried	Professor	In service
6	MOUNDIPA FEWOU Paul	Professor	In service
7	NINTCHOM PENLAP V. épouse BENG	Professor	In service
8	OBEN Julius ENYONG	Professor	In service
9	ACHU Merci BIH	Associate Professor	In service
10	ATOGHO Barbara	Associate Professor	In service
11	AZANTSA KINGUE GABIN BORIS	Associate Professor	In service
12	BELINGA née NDOYE FOE M. C. F.	Associate Professor	*Head DAF / FS*
13	BOUDJEKO Thaddée	Associate Professor	In service
14	DJUIDJE NGOUNOUE Marcelline	Associate Professor	In service
15	EFFA NNOMO Pierre	Associate Professor	In service

16	NANA Louise épouse WAKAM	Associate Professor	In service
17	NGONDI Judith Laure	Associate Professor	In service
18	NGUEFACK Julienne	Associate Professor	In service
19	NJAYOU Frédéric Nico	Associate Professor	In service
20	MOFOR née TEUGWA Clotilde	Associate Professor	*IP Service MINESUP*
21	TCHANA KOUATCHOUA Angèle	Associate Professor	In service

22	AKINDEH MBUH NJI	Lecturer	In service
23	BEBOY EDZENGUELE Sara Nathalie	Lecturer	In service
24	DAKOLE DABOY Charles	Lecturer	In service
25	DJUIKWO NKONGA Ruth Viviane	Lecturer	In service
26	DONGMO LEKAGNE Joseph Blaise	Lecturer	In service
27	EWANE Cécile Anne	Lecturer	In service
28	FONKOUA Martin	Lecturer	In service
29	BEBEE Fadimatou	Lecturer	In service
30	KOTUE KAPTUE Charles	Lecturer	In service
31	LUNGA Paul KEILAH	Lecturer	In service
32	MANANGA Marlyse Joséphine	Lecturer	In service
33	MBONG ANGIE M. Mary Anne	Lecturer	In service
34	PACHANGOU NSANGOU Sylvain	Lecturer	In service
35	Palmer MASUMBE NETONGO	Lecturer	In service

36	MBOUCHE FANMOE Marceline Joëlle	Assistant	In service
37	OWONA AYISSI Vincent Brice	Assistant	In service
38	WILFRIED ANGIE Abia	Assistant	In service

2- DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY (BPA) (46)

1	AJEAGAH Gideon AGHAINDUM	Professor	*Vice-Dean/DSSE*
2	BILONG BILONG Charles-Félix	Professor	*Head of Department*
3	DIMO Théophile	Professor	In service
4	DJIETO-LORDON Champlain	Professor	In service
5	ESSOMBA née NTSAMA MBALA	Professor	*Vice-Dean/FMSB/UYI*
6	FOMENA Abraham	Professor	In service
7	KAMTCHOUING Pierre	Professor	In service
8	NJAMEN Dieudonné	Professor	In service
9	NJIOKOU Flobert	Professor	In service
10	NOLA Moïse	Professor	In service
11	TAN Paul VERNYUY	Professor	In service
12	TCHUEM TCHUENTE Louis Albert	Professor	*IP Service, Progr. Coord. / MINSANTE*
13	ZEBAZE TOGOUET Serge Hubert	Professor	In service

14	BILANDA Danielle Claude	Associate Professor	In service
15	DJIOGUE Séfirin	Associate Professor	In service

16	DZEUFIET DJOMENI Paul Désiré	Associate Professor	In service
17	JATSA BOUKENG Hermine épouse MEGAPTCHE	Associate Professor	In service
18	KEKEUNOU Sévilor	Associate Professor	In service
19	MEGNEKOU Rosette	Associate Professor	In service
20	MONY Ruth épouse NTONE	Associate Professor	In service
21	NGUEGUIM TSOFACK Florence	Associate Professor	In service
22	TOMBI Jeannette	Associate Professor	In service

23	ALENE Désirée Chantal	Lecturer	In service
24	ATSAMO Albert Donatien	Lecturer	In service
25	BELLET EDIMO Oscar Roger	Lecturer	In service
26	DONFACK Mireille	Lecturer	In service
27	ETEME ENAMA Serge	Lecturer	In service
28	GOUNOUE KAMKUMO Raceline	Lecturer	In service
29	KANDEDA KAVAYE Antoine	Lecturer	In service
30	LEKEUFACK FOLEFACK Guy B.	Lecturer	In service
31	MAHOB Raymond Joseph	Lecturer	In service
32	MBENOUN MASSE Paul Serge	Lecturer	In service
33	MOUNGANG Luciane Marlyse	Lecturer	In service
34	MVEYO NDANKEU Yves Patrick	Lecturer	In service
35	NGOUATEU KENFACK Omer Bébé	Lecturer	In service
36	NGUEMBOK	Lecturer	In service
37	NJUA Clarisse Yafi	Lecturer	*Head Division UBa*
38	NOAH EWOTI Olive Vivien	Lecturer	In service
39	TADU Zephyrin	Lecturer	In service
40	TAMSA ARFAO Antoine	Lecturer	In service
41	YEDE	Lecturer	In service

42	BASSOCK BAYIHA Etienne Didier	Assistant	In service
43	ESSAMA MBIDA Désirée Sandrine	Assistant	In service
44	KOGA MANG Debora	Assistant	In service
45	LEME BANOCK Lucie	Assistant	In service
46	YOUNOUSSA LAME	Assistant	In service

3- DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (BPV) (32)

1	AMBANG Zachée	Professor	*Head Division/UYII*
2	BELL Joseph Martin	Professor	In service
3	DJOCGOUE Pierre François	Professor	In service
4	MOSSEBO Dominique Claude	Professor	In service
5	YOUMBI Emmanuel	Professor	*Head of Department*
6	ZAPFACK Louis	Professor	In service

7	ANGONI Hyacinthe	Associate Professor	In service
8	BIYE Elvire Hortense	Associate Professor	In service

9	KENGNE NOUMSI Ives Magloire	Associate Professor	In service
10	MALA Armand William	Associate Professor	In service
11	MBARGA BINDZI Marie Alain	Associate Professor	CT/Minesup
12	MBOLO Marie	Associate Professor	In service
13	NDONGO BEKOLO	Associate Professor	*CE / MINRESI*
14	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	In service
15	TSOATA Esaïe	Associate Professor	In service
16	TONFACK Libert Brice	Associate Professor	In service

17	DJEUANI Astride Carole	Lecturer	In service
18	GOMANDJE Christelle	Lecturer	In service
19	MAFFO MAFFO Nicole Liliane	Lecturer	In service
20	MAHBOU SOMO TOUKAM. Gabriel	Lecturer	In service
21	NGALLE Hermine BILLE	Lecturer	In service
22	NGOUO Lucas Vincent	Lecturer	In service
23	NNANGA MEBENGA Ruth Laure	Lecturer	In service
24	NOUKEU KOUAKAM Armelle	Lecturer	In service
25	ONANA JEAN MICHEL	Lecturer	In service

26	GODSWILL NTSOMBAH NTSEFONG	Assistant	In service
27	KABELONG BANAHO Louis-Paul-Roger	Assistant	In service
28	KONO Léon Dieudonné	Assistant	In service
29	LIBALAH Moses BAKONCK	Assistant	In service
30	LIKENG-LI-NGUE Benoit C.	Assistant	In service
31	TAEDOUNG Evariste Hermann	Assistant	In service
32	TEMEGNE NONO Carine	Assistant	In service

4- DEPARTMENT OF INORGANIC CHEMISTRY (CI) (35)

1	AGWARA ONDOH Moïse	Professor	*Vice-Rector Univ. Ba*
2	ELIMBI Antoine	Professor	In service
3	Florence UFI CHINJE épouse MELO	Professor	*Vice Chancellor Univ. Ndere*
4	GHOGOMU Paul MINGO	Professor	*Minister in Charge of Special Duties P.R.*
5	NANSEU Njiki Charles Péguy	Professor	In service
6	NDIFON Peter TEKE	Professor	*CT MINRESI/Head of Department*
7	NGOMO Horace MANGA	Professor	*Vice Chancellor / UB*
8	NDIKONTAR Maurice KOR	Professor	*Vice-Dean Univ. Ba*
9	NENWA Justin	Professor	In service
10	NGAMENI Emmanuel	Professor	*DEAN FS UDs*

11	BABALE née DJAM DOUDOU	Associate Professor	*In Charge of Special Duties P.R.*
12	DJOUFAC WOUMFO Emmanuel	Associate Professor	In service
13	KAMGANG YOUBI Georges	Associate Professor	In service
14	KEMMEGNE MBOUGUEM Jean C.	Associate Professor	In service
15	KONG SAKEO	Associate Professor	In service
16	NDI NSAMI Julius	Associate Professor	In service
17	NJIOMOU C. épouse DJANGANG	Associate Professor	In service
18	NJOYA Dayirou	Associate Professor	In service
19	YOUNANG Elie	Associate Professor	In service

20	ACAYANKA Elie	Lecturer	In service
21	BELIBI BELIBI Placide Désiré	Lecturer	*C.S / ENS Bertoua*
22	CHEUMANI YONA Arnaud M.	Lecturer	In service
23	EMADACK Alphonse	Lecturer	In service
24	KENNE DEDZO GUSTAVE	Lecturer	In service
25	KOUOTOU DAOUDA	Lecturer	In service
26	MAKON Thomas Beauregard	Lecturer	In service
27	MBEY Jean Aime	Lecturer	In service
28	NCHIMI NONO KATIA	Lecturer	In service
29	NEBA nee NDOSIRI Bridget NDOYE	Lecturer	*CT / MINFEM*
30	NYAMEN Linda Dyorisse	Lecturer	In service
31	PABOUDAM GBAMBIE A.	Lecturer	In service
32	TCHAKOUTE KOUAMO Hervé	Lecturer	In service

33	NJANKWA NJABONG N. Eric	Assistant	In service
34	PATOUOSSA ISSOFA	Assistant	In service
35	SIEWE Jean Mernoz	Assistant	In service
5- DEPARTMENT OF ORGANIC CHEMISTRY (CO) (35)			

1	DONGO Etienne	Professor	*Vice-DEAN / PSAA*
2	GHOGOMU TIH Robert Ralph	Professor	*Dir. IBAF/UDA*
3	NGOUELA Silvère Augustin	Professor	*Head of Department UDs*
4	NKENGFACK Augustin Ephreïm	Professor	*Head of Department*
5	NYASSE Barthélemy	Professor	In service
6	PEGNYEMB Dieudonné Emmanuel	Professor	*Director/ MINESUP*
7	WANDJI Jean	Professor	In service

8	Alex de Théodore ATCHADE	Associate Professor	*DEPE/ Rectorat/UYI*
9	EYONG Kenneth OBEN	Associate Professor	*Head Service DPER*
10	FOLEFOC Gabriel NGOSONG	Associate Professor	In service
11	FOTSO WABO Ghislain	Associate Professor	In service
12	KEUMEDJIO Félix	Associate Professor	In service
13	KEUMOGNE Marguerite	Associate Professor	In service
14	KOUAM Jacques	Associate Professor	In service

15	MBAZOA née DJAMA Céline	Associate Professor	In service
16	MKOUNGA Pierre	Associate Professor	In service
17	NOTE LOUGBOT Olivier Placide	Associate Professor	*Head Service / MINESUP*
18	NGO MBING Joséphine	Associate Professor	*S/Direct. MINERESI*
19	NGONO BIKOBO Dominique Serge	Associate Professor	In service
20	NOUNGOUE TCHAMO Diderot	Associate Professor	In service
21	TABOPDA KUATE Turibio	Associate Professor	In service
22	TCHOUANKEU Jean-Claude	Associate Professor	*DEAN /FS/ UYI*
23	TIH née NGO BILONG E. Anastasie	Associate Professor	In service
24	YANKEP Emmanuel	Associate Professor	In service

25	AMBASSA Pantaléon	Lecturer	In service
26	KAMTO Eutrophe Le Doux	Lecturer	In service
27	MVOT AKAK Carine	Lecturer	In service
28	NGNINTEDO Dominique	Lecturer	In service
29	NGOMO Orléans	Lecturer	In service
30	OUAHOUO WACHE Blandine M.	Lecturer	In service
31	SIELENOU TEDJON Valérie	Lecturer	In service
32	TAGATSING FOTSING Maurice	Lecturer	In service
33	ZONDENDEGOUMBA Ernestine	Lecturer	In service

34	MESSI Angélique Nicolas	Assistant	In service
35	TSEMEUGNE Joseph	Assistant	In service

6- DEPARTMENT OF COMPUTER SCIENCE (IN) (26)			
1	ATSA ETOUNDI Roger	Professor	*Head Div. MINESUP*
2	FOUDA NDJODO Marcel Laurent	Professor	*Head Dpt. ENS/Head IGA.MINESUP*

3	NDOUNDAM Réné	Associate Professor	In service

4	AMINOU Halidou	Lecturer	In service
5	DJAM Xaviera YOUHEP KIMBI	Lecturer	In service
6	EBELE Serge	Lecturer	In service
7	KOUOKAM KOUOKAM E. A.	Lecturer	In service
8	MELATAGIA YONTA Paulin	Lecturer	In service
9	MOTO MPONG Serge Alain	Lecturer	In service
10	TAPAMO Hyppolite	Lecturer	In service
11	ABESSOLO ALO'O Gislain	Lecturer	In service
12	KAMGUEU Patrick Olivier	Lecturer	In service
13	MONTHE DJIADEU Valery M.	Lecturer	In service
14	OLLE OLLE Daniel Claude Delort	Lecturer	*Head Dpt. Enset Ebolowa*
15	TINDO Gilbert	Lecturer	In service
16	TSOPZE Norbert	Lecturer	In service

17	WAKU KOUAMOU Jules	Lecturer	In service

18	BAYEM Jacques Narcisse	Assistant	In service
19	DOMGA KOMGUEM Rodrigue	Assistant	In service
20	EKODECK Stéphane Gael Raymond	Assistant	In service
21	HAMZA Adamou	Assistant	In service
22	JIOMEKONG AZANZI Fidel	Assistant	In service
23	MAKEMBE. S. Oswald	Assistant	In service
24	MESSI NGUELE Thomas	Assistant	In service
25	MEYEMDOU Nadège Sylvianne	Assistant	In service
26	NKONDOCK. MI. BAHANACK.N.	Assistant	In service

7- DEPARTMENT OF MATHEMATICS (MA) (30)			
1	EMVUDU WONO Yves S.	Professor	*Head Dept. Comp. Sci/ Inspector MINESUP*

2	AYISSI Raoult Domingo	Associate Professor	*Head of Department*
3	NKUIMI JUGNIA Célestin	Associate Professor	In service
4	NOUNDJEU Pierre	Associate Professor	In service
5	MBEHOU Mohamed	Associate Professor	In service
6	TCHAPNDA NJABO Sophonie B.	Associate Professor	*Director / AIMS Rwanda*

7	AGHOUKENG JIOFACK Jean Gérard	Lecturer	*Head Service MINPLAMAT*
8	CHENDJOU Gilbert	Lecturer	In service
9	DJIADEU NGAHA Michel	Lecturer	In service
10	DOUANLA YONTA Herman	Lecturer	In service
11	FOMEKONG Christophe	Lecturer	In service
12	KIANPI Maurice	Lecturer	In service
13	KIKI Maxime Armand	Lecturer	In service
14	MBAKOP Guy Merlin	Lecturer	In service
15	MBANG Joseph	Lecturer	In service
16	MBELE BIDIMA Martin Ledoux	Lecturer	In service
17	MENGUE MENGUE David Joe	Lecturer	In service
18	NGUEFACK Bernard	Lecturer	In service
19	NIMPA PEFOUNKEU Romain	Lecturer	In service
20	POLA DOUNDOU Emmanuel	Lecturer	In service
21	TAKAM SOH Patrice	Lecturer	In service
22	TCHANGANG Roger Duclos	Lecturer	In service
23	TCHOUNDJA Edgar Landry	Lecturer	In service
24	TETSADJIO TCHILEPECK M. E.	Lecturer	In service
25	TIAYA TSAGUE N. Anne-Marie	Lecturer	In service

26	MBIAKOP Hilaire George	Assistant	In service
27	BITYE MVONDO Esther Claudine	Assistant	In service

28	MBATAKOU Salomon Joseph	Assistant	In service
29	MEFENZA NOUNTU Thiery	Assistant	In service
30	TCHEUTIA Daniel Duviol	Assistant	In service

8- DEPARTMENT OF MICROBIOLOGY (MIB) (18)

1	ESSIA NGANG Jean Justin	Professor	*DRV/IMPM*

2	BOYOMO ONANA	Associate Professor	In service
3	NWAGA Dieudonné M.	Associate Professor	In service
4	NYEGUE Maximilienne Ascension	Associate Professor	In service
5	RIWOM Sara Honorine	Associate Professor	In service
6	SADO KAMDEM Sylvain Leroy	Associate Professor	In service

7	ASSAM ASSAM Jean Paul	Lecturer	In service
8	BODA Maurice	Lecturer	In service
9	BOUGNOM Blaise Pascal	Lecturer	In service
10	ESSONO OBOUGOU Germain G.	Lecturer	In service
11	NJIKI BIKOÏ Jacky	Lecturer	In service
12	TCHIKOUA Roger	Lecturer	In service

13	ESSONO Damien Marie	Assistant	In service
14	LAMYE Glory MOH	Assistant	In service
15	MEYIN A EBONG Solange	Assistant	In service
16	NKOUDOU ZE Nardis	Assistant	In service
17	SAKE NGANE Carole Stéphanie	Assistant	In service
18	TOBOLBAI Richard	Assistant	In service

9. DEPARTEMENT OF PHYSICS (PHY) (40)

1	BEN- BOLIE Germain Hubert	Professor	In service
2	ESSIMBI ZOBO Bernard	Professor	In service
3	KOFANE Timoléon Crépin	Professor	In service
4	NANA ENGO Serge Guy	Professor	In service
5	NDJAKA Jean Marie Bienvenu	Professor	*Head of Department*
6	NOUAYOU Robert	Professor	In service
7	NJANDJOCK NOUCK Philippe	Professor	*S/Director/ MINRESI*
8	PEMHA Elkana	Professor	In service
9	TABOD Charles TABOD	Professor	*DEAN Univ/Bda*
10	TCHAWOUA Clément	Professor	In service
11	WOAFO Paul	Professor	In service

12	BIYA MOTTO Frédéric	Associate Professor	*DG/HYDRO Mekin*
13	BODO Bertrand	Associate Professor	In service
14	DJUIDJE KENMOE épouse ALOYEM	Associate Professor	In service
15	EKOBENA FOUDA Henri Paul	Associate Professor	*Chef Division. UN*

16	EYEBE FOUDA Jean sire	Associate Professor	In service
17	FEWO Serge Ibraïd	Associate Professor	In service
18	HONA Jacques	Associate Professor	In service
19	MBANE BIOUELE César	Associate Professor	In service
20	NANA NBENDJO Blaise	Associate Professor	In service
21	NDOP Joseph	Associate Professor	In service
22	SAIDOU	Associate Professor	*MINRESI*
23	SIEWE SIEWE Martin	Associate Professor	In service
24	SIMO Elie	Associate Professor	In service
25	VONDOU Derbetini Appolinaire	Associate Professor	In service
26	WAKATA née BEYA Annie	Associate Professor	*S/ Director/* MINESUP
27	ZEKENG Serge Sylvain	Associate Professor	In service

28	ABDOURAHIMI	Lecturer	In service
29	EDONGUE HERVAIS	Lecturer	In service
30	ENYEGUE A NYAM spouse BELINGA	Lecturer	In service
31	FOUEDJIO David	Lecturer	*Head Cell. MINADER*
32	MBINACK Clément	Lecturer	In service
33	MBONO SAMBA Yves Christian U.	Lecturer	In service
34	MELI'I Joelle Larissa	Lecturer	*In service*
35	MVOGO ALAIN	Lecturer	*In service*
36	OBOUNOU Marcel	Lecturer	*DA/ Inter- State Univ /Sangmalima*
37	WOULACHE Rosalie Laure	Lecturer	In service

38	AYISSI EYEBE Guy François Valérie	Assistant	In service
39	CHAMANI Roméo	Assistant	In service
40	TEYOU NGOUPOU Ariel	Assistant	In service

10- DEPARTMENT OF EARTH SCIENCE (ST) (43)

1	BITOM Dieudonné	Professor	*DEAN / FASA / UDs*
2	FOUATEU Rose épse YONGUE	Professor	In service
3	KAMGANG Pierre	Professor	In service
4	NDJIGUI Paul Désiré	Professor	*Head of Department*
5	NDAM NGOUPAYOU Jules-Remy	Professor	In service
6	NGOS III Simon	Professor	DAAC/Uma
7	NKOUMBOU Charles	Professor	In service
8	NZENTI Jean-Paul	Professor	In service

9	ABOSSOLO née ANGUE Monique	Associate Professor	*Vice-Dean / DRC*
10	GHOGOMU Richard TANWI	Associate Professor	*Head Dpt. /UMa*
11	MOUNDI Amidou	Associate Professor	*CT./ MINIMDT*

12	NGUEUTCHOUA Gabriel	Associate Professor	*CEA/MINRESI*
13	NJILAH Isaac KONFOR	Associate Professor	In service
14	ONANA Vincent Laurent	Associate Professor	In service
15	BISSO Dieudonné	Associate Professor	*Dir Memve'ele Dam*
16	EKOMANE Emile	Associate Professor	*In service*
17	GANNO Sylvestre	Associate Professor	In service
18	NYECK Bruno	Associate Professor	In service
19	TCHOUANKOUE Jean-Pierre	Associate Professor	In service
20	TEMDJIM Robert	Associate Professor	In service
21	YENE ATANGANA Joseph Q.	Associate Professor	*Head Div. /MINTP*
22	ZO'O ZAME Philémon	Associate Professor	*DG/ART*

23	ANABA ONANA Achille Basile	Lecturer	*In service*
24	BEKOA Etienne	Lecturer	*In service*
25	ELISE SABABA	Lecturer	In service
26	ESSONO Jean	Lecturer	*In service*
27	EYONG JOHN TAKEM	Lecturer	In service
28	FUH Calistus Gentry	Lecturer	*State Sec. /MINMIDT*
29	LAMILEN BILLA Daniel	Lecturer	In service
30	MBESSE CECILE OLIVE	Lecturer	In service
31	MBIDA YEM	Lecturer	*In service*
32	METANG Victor	Lecturer	In service
33	MINYEM Dieudonné-Lucien	Lecturer	*Head Dept./Uma*
34	NGO BELNOUN Rose Noël	Lecturer	In service
35	NGO BIDJECK Louise Marie	Lecturer	In service
36	NOMO NEGUE Emmanuel	Lecturer	In service
37	NTSAMA ATANGANA Jacqueline	Lecturer	In service
38	TCHAKOUNTE J. épouse NOUMBEM	Lecturer	*Head. cell / MINRESI*
39	TCHAPTCHET TCHATO De P.	Lecturer	In service
40	TEHNA Nathanaël	Lecturer	In service
41	TEMGA Jean Pierre	Lecturer	In service

42	FEUMBA Roger	Assistant	In service
43	MBANGA NYOBE Jules	Assistant	In service

Numerical breakdown of permanent teachers of the Faculty of Science of the University of Yaoundé I

NUMBER OF TEACHERS					
DEPARTMENT	**Professors**	**Associate Professors**	**Lecturers**	**Assistants**	**Total**
BCH	9 (01)	13 (09)	14 (05)	03 (02)	**36 (16)**
BPA	13 (01)	09 (06)	19 (05)	05 (02)	**46 (13)**
BPV	06 (0)	10 (02)	09 (04)	07 (01)	**31 (09)**
CI	10 (01)	09 (02)	13 (02)	02 (0)	**35 (05)**

CO	07 (0)	17 (04)	09 (03)	03 (0)	**35 (07)**
IN	02 (0)	01 (0)	14 (01)	10 (02)	**26 (03)**
MAT	01 (0)	05 (0)	19 (01)	05 (01)	**30 (02)**
MIB	01 (0)	05 (02)	06 (01)	06 (02)	**17 (05)**
PHY	11 (0)	16 (01)	10 (03)	03 (0)	**40 (04)**
ST	08 (01)	14 (01)	19 (04)	02 (0)	**43 (06)**
Total	**68 (4)**	**99 (27)**	**132 (29)**	**45 (10)**	**344 (70)**

Total ----------344 (70) with:
Professors ----------68 (04)
Associate Professors ----------99 (27)
Lecturers ----------132 (39)
Assistants----------46 (10)
() = Number of Women

DEDICATION

I dedicate this thesis to:

My family

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LIST OF ABREVIATIONS

BBTV: Banana bunchy top virus
BRB: Banana root borer
CAGR: Compound annual growth rate
Cc: Center cylinder
DAI: Day after infection
DPI: Day post inoculation
EPA: Environment Protection Agency
EPF: Entomopathogenic fungi
FAO: Food and agricultural organization
GMO: Genetic modified Organism
IITA: International Institute of Tropical Agriculture
IPM: Integrated pest management
MAP: Month after planting
PDA: Potato Dextrose Agar
RH: Relative Humidity
UVB: Ultra violet B
USDA: United States Department of Agriculture
WAI: Week after infestation
WAP: Week after planting
WHC: Water Holding Capacity

ABSTRACT

The production of banana/plantains, a staple food of millions of people in the world is facing a severe parasitic pressure of pests and diseases. Among pests, the banana weevil *Cosmopolites sordidus* is the most damaging insect causing yield loss of up to 100 %. The management of *C. sordidus* is still a challenge. In the effort of managing this pest, several strategies have been used, but the wide spreading of insecticides is still the mainstay. However, public increasing concerns about risk associated with chemicals have led to the use of safe and eco-friendly products such as biopesticides. The objective of this work was to develop a biopesticide formulation from high virulent isolates with wide tolerance to environmental factors against banana weevil *C. sordidus*. Pathogenicity tests of six Cameroonian isolates of *Beauvaeria bassiana* and *Metharizium anisolpliae* were performed on eggs, larvae and pupae of *C. sordidus* as well as endophyte colonization test. The epizootic potential of thermotolerant isolates against *C. sordidus* population was evaluated using pathogenicity test at proportion 10. 30, and 50% vector ratio.Germination, mycelial growth and conidia production was also determine after culture entomopathogenic fungi under divers environmental conditions. Theselection of quality control, compatibility and photoprotection tests for 12 local ingredients.. The mycoinsecticide formulations developed were applied alone or combined with aggregation pheromone Cosmolure, in the laboratory, greenhouse, and natural environment, and mortality and infections rates were determine.

The results of insect life stages susceptibility to entomopathogenic fungal isolates showed that the infection rates depended on the insect's life stage and isolates which varied from 12 - 96 %, 60 - 100 %, and 12.50 - 79.44 % respectively for eggs, 5-instar larvae, and pupae. Apart from being pathogenic and virulent to multiple stages, these isolates transmitted disease from one life stage to the next.

The thermotolerant Beauveria isolates, BIITAC6.2.2 and BIITAC10.3.3, Metarhizium isolates MIITAC6.2.2 and MIITAC11.3.4 caused horizontally of up to 52 % mortality of non-inoculated insects at vector ratio of 50 %, with high values for the isolate BIITAC6.2.2 followed by MIITAC11.3.4.

Environmental factors such as pH, photoperiod, UV radiations, and relative humidity affected significantly the development of Cameroonian entomopathogenic fungi isolates on artificial media. The fungi strain MIITAC11.3.4 and BIITAC6.2.2 tolerated acidic, near neutral, and alkaline conditions. the optimum growth was obtained at pH range 4 – 11 and 4 - 12 respectively for MIITAC11.3.4 and BIITAC6.2.2. BIITAC6.2.2 is less exigent to light and can be suitable for control of insects of all habitat while MIITAC11.3.4 gave optimum development in total dark and 12L:12D. The two isolates tested in this study were found tolerant of UVB-light. The UVB-light delayed conidia survival, and at the same time, stimulated conidia production. The fungi isolate MIITAC11.3.4 was more stable while BIITAC6.2.2 produced a mutant after 30 min exposure to UVB-light. MIITAC11.3.4 and BIITAC6.2.2 could also grow at low to high humidity environments but a moderate humidity environment was preferable for their best expression.

Mass-produced conidia of *Beauveria bassiana* BIITAC6.2.2 showed high quality. Out of the twelve inactive ingredients tested four were found to be the best carriers of BIITAC6.2.2 conidia (kaolin clay, cassava starch, maize flour, and banana flour), two good stabilizer (charcoal and wood ashes) and four effective UV protectants (kaolin clay, cassava starch, banana flour, and charcoal.

The potential of the four formulations developed varied from one condition to another. Under laboratory conditions, formulations conserved good stability at 4 °C, -20 °C, and -50 °C for up to 72 weeks and enhanced insecticidal potential of active ingredient (causing up to 100 % mortality). Results of the greenhouse trial indicated that *B. bassiana* BIITAC6.2.2 formulations can be used both as prevention (53.33 - 68.67 % mortality) and post-infestation (66 - 84.67 % mortality) methods. When applied alone in the field, mycoinsecticide did not show a clear positive effect for the first crop cycle. However, two other banana pests (*Pollytus mellerborgi* and unidentified insect) showed signs of mycosis apart from *C. sordidus*. The preliminary study of the performance of mycoinsecticide combined with pheromone baited traps revealed that the pheromone trap can optimize mycoinsecticide efficacy in the field, causing up to 83.3 and 78.7 % mortality and mycosis rates of adult *C. sordidus*.

Keywords: banana weevil, banana crop, entomopathogenic fungi, mycoinsecticide, performance, integrated pest management.

RESUME

La production de bananes / plantains est confrontée à une grande pression parasitaire et aux ravageurs. Parmi les ravageurs, le charançon du bananier *Cosmopolites sordidus* est l'insecte le plus nuisible pouvant entraîner des pertes pouvant atteindre 100 %. La gestion de *C. sordidus* reste un défi. Dans le cadre de la lutte contre ce ravageur, plusieurs stratégies ont été utilisées, mais la diffusion à grande échelle d'insecticides en est toujours le pilier. Cependant, la préoccupation du public sur les risques liés aux produits chimiques, a conduit à la recherche de produits non-dangereux et écologiques tels que les biopesticides. L'objectif de ce travail était de développer une formulation de biopesticide à base d'isolat virulent à large spectre de tolerance aux facteurs environementaux dans le cadre de la lutte contre le charançon du bananier. Les tests de pathogenicités de six isolats de champignons entomopathogènes du Cameroun ont été effectués sur les eggs, larves et pupes de *C. sordidus* ainsi que le test de colonization endophytique. Le potentiel épizootique des isolats thermotolérants dans une population de *C. sordidus* a été evalué gråce au test de pathogenité au proportion 10, 30, et 50% de charancons vecteur. La germination, de la croissance mycelienne et la production conidienne ont été egalement determiné après cultures des champignons entomopathogènes sous divers conditions environnementaux. La sélection des ingrédients principaux à intéger dans la formulation des mycoinsecticides s'est fait en effectuant les tests de control de qualité, de compatibilité et de photoprotection à partir 12 ingredients locaux. Les formulations de mycoinsecticides developpés ont été appliqués seuls ou combinés avec la phéromone d'agrégation Cosmolure, au laboratoire, en serre et en milieu naturel contre *C. sordidus* et les taux mortalités et d'infections ont été determinés.

Les resultats de la sensibilité des stades de development aux isolats fongiques entomopathogènes, ont montré une pathogenicité et une virulence dépendante des stades de vie de *C. sordidus* et des isolats avec le taux d'infection combinés compris entre 12 à 96 %, 60 à 100 % et 12.50 à 79.44 % respectivement pour les œufs, les larves de stades 5 et les pupes. En plus d'être pathogènes et virulents à plusieurs stades, ces isolats ont transmis la maladie d'un stade de vie au suivant.

Les isolats thermotolerant de Beauveria, BIITAC6.2.2 et BIITAC10.3.3, Metarhizium MIITAC6.2.2 et MIITAC11.3.4 ont causé horizontalement jusqu'à 52% mortalité d'insectes non inoculés lorsque 50 % d'insectes sont porteur d'inoculum, avec des valeurs élevées pour l'isolat BIITAC6.2.2 suivi de MIITAC11.3.4.

Les facteurs environnementaux tels que le pH, photopériode, les radiations UV et humidité relative ont affectés de manière significative le développement d'isolats de champignons

entomopathogènes du Cameroun sur milieu artificiel. Les isolats MIITAC11.3.4 et BIITAC6.2.2 ont toléré des conditions acides, quasi neutres et alcalines. La croissance optimale a été obtenue dans la gamme de pH 4-11 et 4-12 respectivement pour MIITAC11.3.4 et BIITAC6.2.2. BIITAC6.2.2 a été moins exigeant en lumière et peut convenir au contrôle des insectes de tous les habitats tandis que MIITAC11.3.4 a donné un développement optimal dans l'obscurité totale et 12 h eclairage: 12 h obscurité. Les deux isolats testés dans cette étude se sont révélés tolérants au UV. La lumière UVB a retardé la survie des conidies et en même temps stimulé la production de conidies. L'isolat de champignon MIITAC11.3.4 est plus stable tandis que BIITAC6.2.2 peut produire un mutant après 30 min d'exposition à la lumière UVB. MIITAC11.3.4 et BIITAC6.2.2 s'est également développé dans un environnement d'humidité faible à élevée, mais un environnement d'humidité modérée s'est avéré préférable pour leur meilleure developpement.

Les conidies produites en masse de Beauveria BIITAC6.2.2 ont montré une qualité élevée. Quatre ingredients inactifs se sont avérés être de bon porteurs de conidies. Il s'agit de l'argile de kaolin, l'amidon de manioc, la farine de maïs et la farine de banane. Le charbon de bois et la cendre de bois ont été de bons stabilisants tandis que le kaolin argile, l'amidon de manioc, la farine de banane et le charbon de bois ont été efficace dans le protection contre les UV.

Le potentiel des quatre formulations développées variait d'une condition à l'autre. En conditions de laboratoire, les formulations ont conservé une bonne stabilité à 4 °C, -20 °C et -50 °C pendant 72 semaines et un potentiel insecticide accru de l'ingrédient actif (provoquant jusqu'à 100 % de mortalité). Les résultats de l'essai en serre ont indiqué que les formulations de *B. bassiana* BIITAC6.2.2 peuvent être utilisées à la fois en méthode préventive (mortalité 53,33 - 68,67 %) et post-infestation (mortalité 66 - 84,67 %).

Lorsque appliqué seule en champ, le mycoinsecticide n'a pas montré d'effet positif clair pour le premier cycle de culture. Cependant, deux espèces d'insectes nuisibles du bananier (*Pollytus mellerborgi* et insecte non identifié) ont montré des signes de mycose en plus de *C. sordidus*. L'étude préliminaire des performances du mycoinsecticide combiné aux pièges phéromones a révélé une optimisation de l'efficacité des mycoinsecticides sur le terrain, provoquant jusqu'à 83,3 et 78,7 % de mortalité et de mycose chez les adultes de *C. sordidus*.

Mots clés: Charançon, bananier, champignons entomopathogènes, mycoinsecticides, performance, lutte intégrée.

INTRODUCTION

Banana and plantain (*Musa* spp.) are important food crops worldwide. They represent the fourth most important global food commodity after rice, wheat, and maize in terms of the gross value of production(Frison et Sharrock, 1998). The richness of their fruit in vitamins and carbohydrates has given them vital importance for food security for more than 400 million people in the world. About 187 million metric tons of banana and plantain are produced annually worldwide by 138 countries (FAO, 2018). Cameroon (5.14 million tons) is the 8th producer worldwide and the biggest producer in Central Africa and Africa (FAO, 2018). Despite the high economic importance of banana/plantain for Cameroon, the cultivation of this crop is a challenge. Banana yield is significantly reduced due to several diseases and pests pressure associated with poor soil fertility (Swennen *et al.*, 2013).

Banana weevil (*Cosmopolites sordidus),* is the most important insect pest of banana for its destructive potential and its famous presence in almost all the part of the world(Gold *et al.*, 1994; Okolle *et al.*, 2009). In Cameroon, they are found in all areas of banana and plantain production (Okolle *et al.*, 2009). *C. sordidus* have been responsible for the reduction of planting material availability through premature dead of suckers; the decline of productivity by reducing bunches sizes and banana plants falling resulting in a reduction of plantation lifespan (Gold *et al.*, 2001). Banana weevil activities had led to yield loss of up to 100 % in a condition of severe infestation with low or absence of management strategies (Sengooba, 1986; Koppenhofer *et al.*, 1994; Muñoz *et al.*, 2013). This has surely contributed to the increase of banana or plantain prices in national, regional, and local markets. Several strategies have been used in the banana farming system for the management of banana weevil, including cultural practices, genetic, chemical and biological methods (Kiggundu *et al.*, 2003b; Akmal *et al.*, 2013; Arinaitwe *et al.*, 2015; Mongyeh *et al.*, 2015; Tinzaara *et al.*, 2015; Sivirihauma *et al.*, 2017). Up to date pesticide remains the most used tool for solving the problem of crop pests including *C. sordidus* population reduction, despite the knowledge on adverse effects such as the development of resistance to pest population, action on the beneficial organism (biodiversity), presence of residues, effect on human health and environmental pollution (Nicolopoulou-Stamati *et al.*, 2016; Kim *et al.*, 2017; Carvalho, 2017).

The consciousness on the deleterious effect of the chemical has led to a return to safe control strategies such as microbial biopesticides use like bioinsecticide based on bacteria (Ambang *et al.*, 2002), plant extract (Ambang *et al.*, 2005; Mboussi *et al.*, 2018) and fungi

(Nankinga et *al.*, 1999). The two promising entomopathogenic fungi belonging to the class of Deuteromycota, sup class of Hyphomycetes, *Beauveria bassiana* and *Metarhizium anisopliae* are the most used for the control of several agricultural pests, including *C. sordidus* (Nankinga *et al.*, 1999; Schoeman and Schoeman, 1999; Aby *et al.*, 2010; Lopes *et al.*, 2011; Tinzaara *et al.*, 2015). Several works have been carried out in many countries under laboratory and field conditions showing mortality on pests up to 100 % (Nankinga and Moore, 2000; Godonou *et al.*, 2000, Lopes *et al.*, 2011; Fancelli *et al.*, 2013; Lopes *et al.*, 2014). The reduction of fungi effectiveness in the field conditions is related to fungi survival, fungi virulence, adaptability in specific conditions, formulations, an interval of time for biopesticide application and implementation technology (Vanninien *et al.*, 2000; Magara *et al.*, 2003; Aby *et al.*, 2010; Fancelli *et al.*, 2013; Moreira *et al.*, 2017; Gonzalez *et al.*, 2018; Membang *et al.*, 2020;). Commercial products (Botanigard, Ostrinil…) based on these fungi are readily available for the control of various species of agricultural pests (Akmal *et al.*, 2013) but many products have been withdrawn from the biopesticide market due to ecological and environmental challenges (Aby *et al.*, 2010, Maina *et al.*, 2018). The problem of adaptability in local conditions and specificity of the host has limited the use of many biopesticide formulations from a specific virulent strain. For example, Botanigard formulated from strain GHA has shown performance in the country where the strain was isolated and other countries related climate. The same biopesticide tested in Cameroon has caused only 25 % mortality on adult *C. sordidus (*Okolle *et al.*, 2009) whereas local isolates caused up to 92 % mortality (Fogain, 1994). This adaptability problem may be related to climate changes and genetic diversity. Membang (2013) isolated 40 isolates from the banana rhizosphere using a baited method with *C. sordidus* larvae. The potential of Cameroonian entomopathogenic fungal isolates are yet not fully exploited, no information exists on their performance as biopesticide formulation in the field. The development of an effective biopesticide formulation involved several steps from the selection of suitable strain to the application method in the field. This ability of horizontal transmission has become a decisive factor for disease evolution in the pest population (Lopes *et al.*, 2011).

In this study we shall answer the following research questions:

- What are the potentials of Cameroonian isolates of entomopathogenic fungi against *C. sordidus*?

- What is the epizootic potential of thermotolerant Cameroonian isolates of entomopathogenic fungi?
- What is the impact of environmental factors on entomopathogenic fungi development *in vitro?*
- Which local ingredients are suitable for mycoinsecticide formulation?
-What are the performances of mycoinsecticides formulation in laboratory, greenhouse, and field conditions?

The development of an effective biopesticide formulation as an alternative to pesticides is of great importance since it can contribute to plant and environment protection and as well as enhance banana and plantain production. Four hypotheses can be formulated from the current study**:**

- H1: Cameroonian entomopathogenic fungal isolates have multiple functions depending on *C. sordidus* life stages;
- H2: The thermotolerant Cameroonian isolates have similar epizootic potential on *C. sordidus*;
- H3: The development of entomopathogenic fungi *in vitro* is significantly affected by environmental factors.
- H4: Cameroonian isolates have a large list of promising local ingredients for mycoinsecticide formulation development;
- H5: mycoinsecticide formulations are highly effective in the lab, greenhouse, and field

The overall objective of this study is to develop a biopesticide from high virulent isolates with wide tolerance to environmental factors.

The specific objectives of this study are to:

1- assess efficacy of entomopathogenic fungi isolates against *C. sordidus* life stages and endophytic colonization;
2- determine the epizootic potential of thermotolerant fungi isolates in *C. sordidus* population;
3- evaluate the effect of some environmental factors on the development of entomopathogenic fungi *in vitro*;
4- Select major ingredients to be integrated with biopesticide formulation;

5- Characterize mycoinsecticide formulations in the laboratory, greenhouse, and natural environment.

The work presented in this thesis combines studies on seasonal polyphenism, temperature induced plasticity and thermal stress adaptation in relation with *B. dorothea*. At the start of this work, an introduction gives the context and state the rationale of this study. In the first major part, a literature review gives all information on the actual knowledge on banana as crop, *C. sordidus*, systematic, origin and distribution, ecology and biological cycle, management strategies and biopesticides as pest management tool. The second part details the methods and the material used to achieve this study, followed by a section on results and discussion. This thesis concludes with highlights of salient results and recommendations.

CHAPTER I. LITTERATURE REVIEW

I.1. Banana as crop

I.1.1. Botanical description

Banana is a perennial, monocotyledonous herb of 1.5 – 6 m long; some cultivars can reach 7 - 15 m height. Plantains pseudo-stem are often larger than those of bananas. The "trunk" or pseudo-stem is not a true stem, but a clustered and cylindrical aggregation of leaf stalk bases (fig. 1A). Leaves are among the largest of all plants, with up to 9 feet long and 2 feet wide. Margins are entire, and venation is pinnate; leaves tear along the veins in windy conditions, giving a feathered or tattered look. There are 5 - 15 leaves on each plant, with 10 leaves considered as the minimum for properly maturation of a bunch of fruit (fig. 1B). The perennial portion of the plant is the rhizome (fig. 1C), which can weigh several kilograms. The rhizome is an important storage organ that sustains the growth of the bunch (fig. 1C) and the development of the suckers. The sucker's production depends on the internal (ploidy level, genomic constitution, and age of the mother plant) and external (planting season, plant density, and plant depth) factors (Blomme *et al.*, 2000).

I.1.2. Origin of banana

Musa species (spp.) are not native to Africa (Blomme *et al.*, 2013). Their origin is very controversial. Nevertheless, Asia was recognized as the center origin of *Musa* species precisely in the territories of actual Malaysia and South-East India (Blomme *et al.*, 2013). It is there that the earliest domestication occurred and continued via vegetative propagation and the natural crossing of cultivated plants grown near various genotypes of *M. accuminata* (AA genome) and *M. balbisiana* (BB genome) (Simmonds, 1966; Perrier *et al.*, 2009). It is hypothesized that they would have reached for the first time Africa through Zanzibar in Tanzania between the 6th and 7th centuries and would have spread to West Africa with the migration of Bantu (Swennen and Vuylsteke, 2001) through the transportation planting material from one place to another. Colonial farmers, travelers, missionaries, and migrants have also played an important role in the introduction and dissemination of new banana cultivars from various botanical gardens (Blomme *et al.*, 2013). Currently, West and Central Africa are identified as a secondary center of diversification of plantain with more than 100 cultivars (Norgrove and Hauser, 2014) and thus has the world's highest diversity (Blomme *et al.*, 2013).

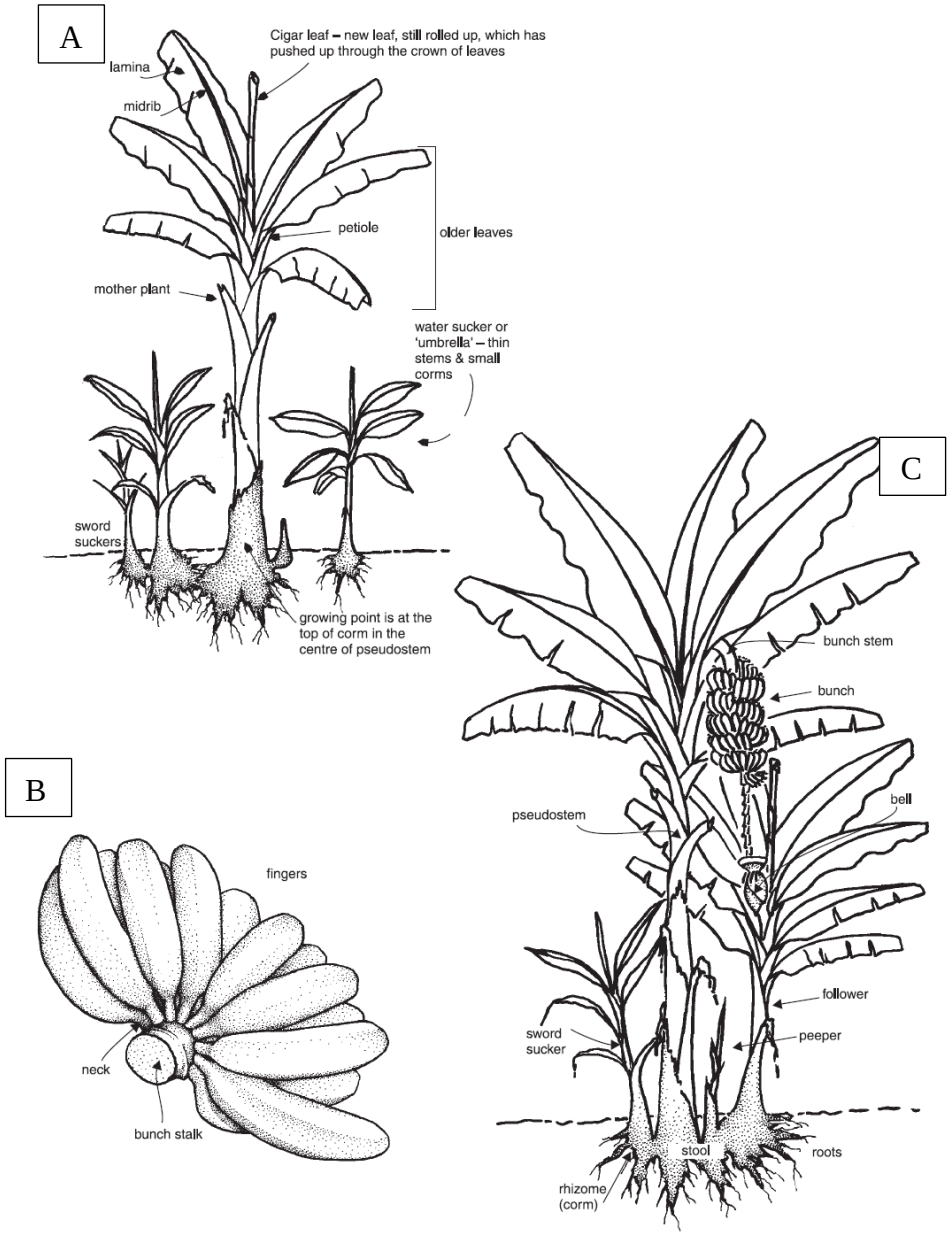

Fig. 1. Morphological description of banana crop (Broadley, 2004); A= banana stool-vegetative stage; B= hand of banana; C= banana stool-reproductive stage.

I.1.3. Taxonomy and genetics of banana crop

The banana crop is a monocotyledon that belongs to the Order of Zingiberales and the family of *Musaceae*. This family has two genera *Musa* and *Ensete* (Champion, 1976). The

genus *Musa* has around 25 - 80 species (depending on the taxonomist) divided into five sections:

sections *Eumusa* (2n= 22 chromosomes); sections *Rhodochlamys* (2n= 22 chromosomes) ; sections *Australimusa* (2n= 20 chromosomes) ; sections *Callimusa* (2n= 20 chromosomes) ; and section *Ingentimusa* (2n= 28 chromosomes).

The more cultivated bananas belong to *Eumusa* group, where the most geographically widespread species are found. Most cultivars are derived from two species, *Musa acuminata* (AA) and *Musa balbisiana* (BB) (fig.2).

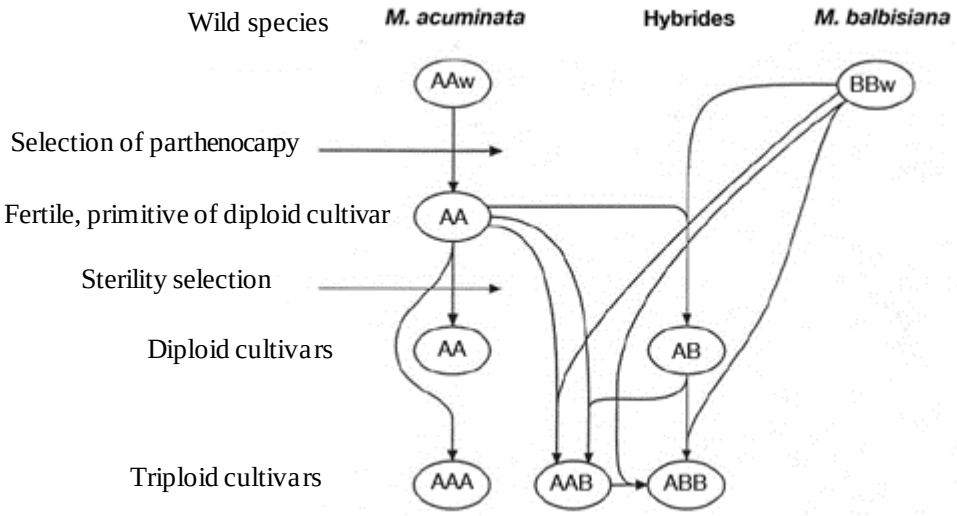

Fig. 2. Evolution of the main genomic groups of edible banana cultivars of the *Eumusa* series; W: wild type (Jones, 2000).

The genus *Musa* includes both seed and non-seed (parthenocarpy) types (fig. 2). The seed type, wild type, are diploid and can be multiplied by sexual (strict allogamy) and asexual reproduction. Cultivated banana is obtained from hybridization between *M. acuminata* and *M. balbisiana* (Simmond, 1962). They are multiplied only in a vegetative way. Genomic group results from the crossing between *M. acuminata* and *M. balbisiana* (Bakry *et al.*, 1997)*:*

- diploid groups AA and AB encompass varieties such as “Figue Sucrée” or “fressinette”. There are 290 cultivars known mainly in South-East Asia;
- triploid group AAA comprises the dessert banana varieties: Gros Michel, “Figue Rose” and Cavendish sub-group (more than 20 cultivars);
- The triploid group AAB comprises cooking banana varieties, such as plantains (more than 150 cultivars) and other cooking cultivars;

- The triploid group ABB comprises hardy, robust cooking banana varieties (e.g.: Bluggoe or Cacambou, Saba, Pisan Awak or Fougamou, Pelipita, etc.);
- tetraploid group (AAAA, AAAB, AABB, ABBB), example AABB cooking type which had the highest average annual yield, followed by 'FHIA-17', an AAAA Gros Michel type dessert banana (MusaNet, 2016).

Five types of local varieties are found in Cameroon: type « French » (*Essong, Ovang, Elat, Njock kon; Alou vini*; *Mekintu*), false horn type (*Maliwa ma Liko*; *Big Ebanga*; *Ebang*; Bouroukou), type True horn (Mota mo Liko) and recently Batard (French horn). Improved varieties such as *Fougamou, Pisang, PITA 24, PITA 21, FHIA 03, CRPB, PITA21, PITA14, FHIA25,* and *FHIA21* are also found in Cameroon.

I.1.4. Ecological requirements

The banana tree is a high demand water plant, which requires rainfall of at least 1300 - 3600 mm water/year according to the evapotranspiration rate (Du Montcel, 1985). When the quantity of rainfall is not adequate, an irrigation system can be recommended.

Banana and plantain are generally cultivated under a warm subtropical climate at temperature ranges between 26 - 30 °C (Newley *et al.*, 2008) and relative humidity between 74 – 80% (Salau *et al.*, 2016).

Apart from salty soils, banana trees grow in almost all types of soils. They prefer slightly acid soils with a pH ranging between 5.5 and 6.5, well-drained, deep, loamy, loose and aerated, rich in organic matter and mineral elements. The elements N, P, K, Ca, and Mg are essential for good development and high production in bananas (Swennen and Vuylteke, 2001). Uncultivated soils are generally preferable because of the abundance of organic matter as well as fallow, but the yield is dependent on cropping history and length of fallow. Soil moisture of 80 to 100 % of the field capacity is required for good growth of the banana, corresponding to a rainfall of 100 mm/month (Swennen and Vuylteke, 2001).

I.1.5. Socio-economic and nutritional importance of banana/plantain

I.1.5.1. Banana and plantain production

Banana and plantain (*Musa* spp) figure among the top ten food crops worldwide. They occupied the rank of the fourth most important global food commodity after rice, wheat, and

maize in terms of the gross value of production (Frison and Sharrock, 1998). Banana and plantain play vital importance for food security for more than 400 million people in the world to whom they constitute a staple food. In Africa, *Musa* spp. provide more than 25 % of the carbohydrate requirements for over 70 million people (Asif and Kaur, 2017). Banana and plantain are cultivated in 151 countries of the tropical and sub-tropical regions, on over a harvested area of approximately 11.37 million hectares, with an annual production of around 158.3 million metric tons (FAO, 2019). The biggest banana producer is India, with 30.4 million tons followed by China (11.22 million tons). Cameroon produces only 3.78 % (5.98 million tons) of the worldwide annual banana production and is therefore ranked 8 th producer worldwide. In Africa, Cameroon is the biggest producer of banana and plantain followed by the Congo Democratic Republic (5.66 million tons) (FAO, 2019).

In Cameroon, the area under plantain and banana cultivation represents 63 % of the national area, principally in seven meridional regions of the country: Center, East, Littoral, Nord-west, West, South-west, and South regions (Bikoï, 1999).

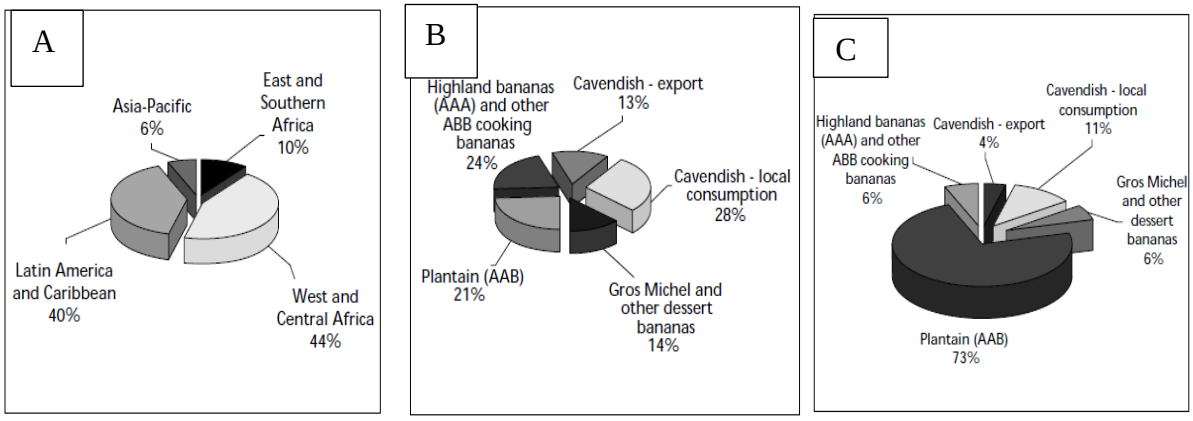

Fig. 3. Major types of banana grown worldwide and Musa production in West and Central Africa (Frison and Sharrock, 1998). A= regional production; B= genotype produced in West Africa and C= genotype produced in Central Africa

I.1.5.2. Banana as exported crop

Asia is the largest banana-producing region while Latin American and the Caribbean are the largest exporting region with shipments of approximately 80% of global exports (IISD,

2020). Asian countries exported 22.46 % of global banana exportation while African exported 3.93 % (FAO, 2019).

In 2019, the main exporting countries were Ecuador, the Philippines, Guatemala, Colombia, and Costa Rica, while the main importers of the banana were United States, China, the Russian Federation, Japan, and Canada (FAO, 2019).

I.1.5.3. Banana social and nutritional importance

Although banana and plantain are present in international markets, they are produced in the vast majority by smallholders for either home consumption or local markets. The highest per capita consumption of banana/plantain is for Uganda, Rwanda, and Cameroon with over 200 kg/year, especially in rural areas (IISD, 2020)

Bananas provide a good source of nutrients for both human and animal consumption It is useful in food, feed, pharmaceutical, packaging, and many other industrial applications. Considering the nutritional aspects, it is one of the world's leading food crops with a great source of minerals (macro-elements: sodium, potassium, calcium, iron, magnesium and phosphorus and oligo-elements: iodine, copper, fluorine, chloride, zinc, cobalt, selenium and manganese), vitamins (Vitamin E, Vit B1, Vit B2, and Vit C), carbohydrates, flavonoids, phenolic compounds (Asif and Kaur, 2017). They are consumed ripe or unripe as cooked and uncooked form. Cooking bananas and plantains are generally eaten boiled, steamed, fried, or roasted while sweet dessert bananas are eaten raw (fruit). Banana plant parts are useful as an insecticide, metal chelating power, reducing power, antioxidant, color absorber, in preparation of various functional foods, wine, alcohol, biogas, cattle feed, flour, starch preparation, bread making, ornamental function, etc (Asif and Kaur, 2017). Musa species have been reported to have various biological activities like antiulcerogenic, antidiabetic, antiatherogenic, antidiarrheic, antitumoral, antimutagenic, migraine, hypertension, cholesterol, and hyperoxaluric (Asif and Kaur, 2017). Banana leaves and fiber are used for handicrafts, ropes, and wrapping foodstuff while banana peels are served for animal feeding (Frison and Sharrock, 1999).

I.1.6. Constraints of banana production

Banana yield is significantly reduced due to several diseases and pests pressure associated with poor soil fertility (Karamura *et al.*, 1999; Stover, 2000; Viljoen *et al.*, 2017).

Diseases and pests are responsible for great damages according to variety and all parts of the plants can be affected during the plant cycle. Fungus, bacteria, and viruses are usually the cause of diseases observed on banana plants (Jones, 2000a).

I.1.6.1. Banana diseases

Banana is attacked by the following diseases: yellow Sigatoka, black Sigatoka, and Sigatoka leaf spot caused respectively by *Mycospharella musicola*, *M. fijiensis* and *M. eumusae* (Khan *et al.*, 2015; Panama disease or Fusarium wilt by *Fusarium oxysporum f. sp. Cubense* (Blomme *et al.*, 2017) and post-harvest rots usually anthracnose (*Colletotrichum musae*), crown rot (*Fusarium semitectum* or *F. musae*), Finger rot (*Lasiodiplodia theobromae),* and cigar end rot (*Verticillium theobromae*) (Triest and Hendrickx, 2016; Jagana *et al.*, 2017)

According to Blomme *et al* (2017), Bacterial diseases of banana and Enset can be classified into three distinct groups: a) Ralstonia-associated diseases (Moko/Bugtok disease caused by *Ralstonia solanacearum* and banana blood disease caused by *R. syzygii* subsp. *celebesensis*); b) *Xanthomonas* wilt disease caused by *Xanthomonas campestris* pv. *musacearum* and c) *Erwinia*-associated diseases (bacterial head rot or tip-over disease (*Erwinia carotovora* ssp. *carotovora* and *E. chrysanthemi*), bacterial rhizome and pseudo-stem wet rot (*Dickeya paradisiaca* formerly *E. chrysanthemi pv. paradisiaca*).

Banana is known to be affected by five viral diseases: a banana mosaic of infectious chlorosis caused by Cucumber mosaic virus (CMV) transmitted by several aphid species (e.g. *Aphis gossypi, Myzus persicae*) naturally found on a very wide range of plant species, including cultivated crops (e.g. tomato, pepper, cucurbits) and weeds; banana bunchy top disease by the banana bunchy top virus (BBTV) transmitted by a single aphid species *Pentalonia nigronervosa*, black banana aphid (Ngatat *et al.*, 2017); banana streak disease by Banana streak virus (BSV) which can be transmitted by mealybugs; Bract mosaic caused by banana bract mosaic virus (BBrMV) transmitted in a non-persistent manner by at least three species of aphid, *Aphis gossypi, Rhopalosiphum maidis* and *Pentalonia nigronervosa;* and Banana mild mosaic diseases by Banana mild mosaic virus (BanMMV) transmitted by vegetative propagation *(*Teycheney *et al.*, 2007*).*

I.1.6.2. Banana pests

Yield losses associated with banana pests can reach up to 100 % (Okolle *et al.*, 2009; Munoz *et al.*, 2013). They attack pseudo-stem, bulbs, roots, leaves, and very rarely fruits. Mealybugs, nematodes, and weevils are pests of bananas (Okolle *et al.*, 2009).

There are several types of nematodes attacking banana: Root-knot nematodes (*Meloidogyne* spp.) which enter the root tissues of the plant and form giant cells; lesion nematodes (*Pratylenchus coffeae* and *P. goodeyi*) that reduce the root system; and root-knot nematodes (*Radophilis similis*) preferentially feeding on the cytoplasm of the cells of the cortex (Kosma *et al.*, 2012). These different types of nematodes are responsible for the disruption of xylem functioning and hydromineral absorption, stunting of plants, reduction of the weight of the diet, and plant shedding under wind or heavy rainfall (Jones, 2000a).

Banana mealybug such as *Pseudococcus* spp., *Geococcus* spp., *Cataenococcus ensete* are scale insects that attack the banana bulb at all ages (Addis *et al.*, 2008). Their dispersal is facilitated by the movement of infested plant releases, cultivation in infested fields, repeated transplant operation, and association with ants (Addis *et al.*, 2008). These mealybugs are the cause of banana growth delay and lethal leaf dehydration.

Studies in all banana growing areas in Cameroon (Okolle *et al.*, 2009) have identified several species of weevils. Ysenbrandt *et al* (2000) mention the presence of *Metamasius sericeus*, *M. hemipterus* L. (Squire 1972), *Pollytus mellerborgi* Boheman, and *C. sordidus* Germar. Apart from those mentioned above, there are others in the world namely: *C. pruinosus* (Musanza *et al.*, 2003), *Temnoschiota nigroplagiata* (Qued), *T. erudita* Duviv., *T. basipennis* Duviv (Harris, 1947), and *Oidoporus longicollis* L. (Adisoemarto, 1983); but the banana weevil *C. sordidus* is the most known and the most disastrous banana pest arthropod that needs to be controlled (Ysenbrandt *et al.*, 2000, Musanza *et al.*, 2003, Okolle *et al.*, 2009).

I.2. Banana weevil, Cosmopolites sordidus

I.2.1. Systematic position

Cosmopolites sordidus was first described as *Calandra sordida* by Ernst Fredreich Germar in 1824. It was named *Cosmopolites sordidus* by Chevrolat in 1885. Several synonyms are used to call this insect: banana weevil borer, banana root weevil, banana root borer, banana rhizome weevil, banana borer, plantain weevil, corm weevil, black weevil of banana, and banana beetle. It belongs to the kingdom Animalia, phylum Arthropoda, class

Insecta, order Coleoptera, family Dryophthoridae, tribe Sphenophorni, genus Cosmopolites, and binomial *Cosmopolites sordidus*.

I.2.2. Origin and distribution

Cosmopolites sordidus is native to the Indo-Malaysian zone (Musanza *et al*., 2003). It has spread through the exchange of infected planting materials and the displacement of adults capable of colonizing plantations established nearby infested fields (Seshu Reddy *et al*., 1999). Currently, the presence of *C. sordidus* is reported in almost all banana production areas (CABI,2020) (fig.4). In Cameroon, it is found in all areas of banana and plantain production (Okolle *et al.*, 2009).

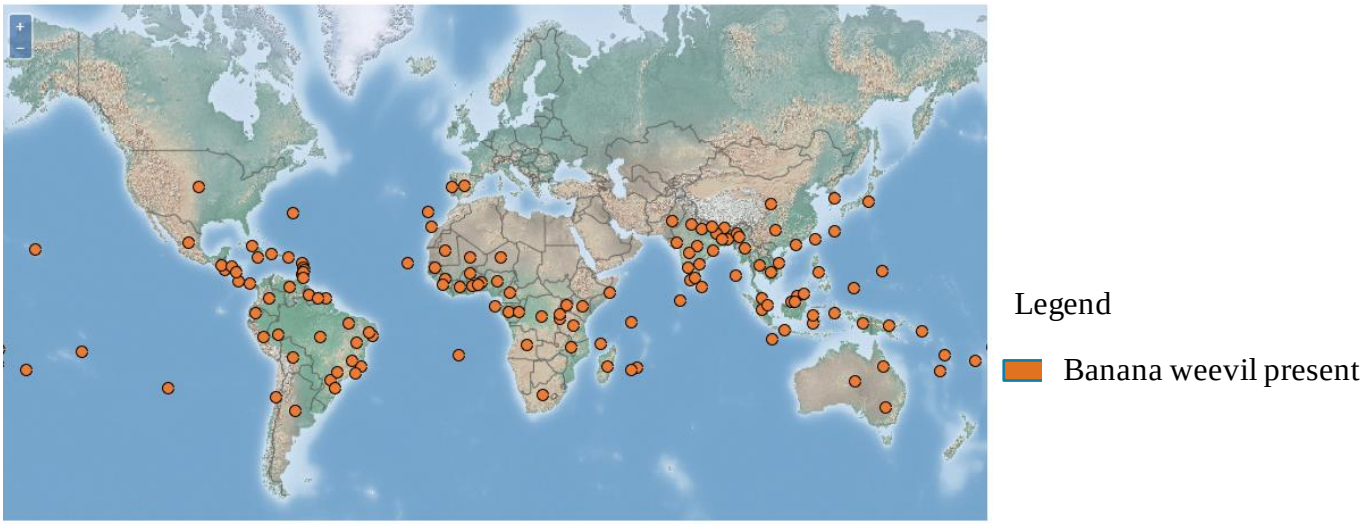

Fig. 4. Wordl distribution of banana weevil (CABI, 2020)

I.2.3. Ecology and biological cycle of *Cosmopolites sordidus*

Cosmopolites sordidus is a polyphagous species reported as host to Euphorbiaceae (*Ricinodendron hendelotii*), Gramineae (*Saccharum* sp and *Panicum maximum*), Araceae (*Xanthosoma sagittifolia*), and Dioscoraceae (*Dioscorea batatas*) (Pavis, 1988). It is one of the main pests of Musa sp (banana and plantain) and Ensete spp. (ensete). The highland cultivars are particularly susceptible to this pest (Kiggundu *et al*., 2003).

Banana weevils can attack all vegetative stages of banana (flowering stage, fruiting stage, seedling stage, and vegetative growing stage), and the parts affected are corm, stems, roots, (in case of severe infestation), and the harvested stumps (Okolle et al., 2009).

Cosmopolites sordidus can also be found in the soil at the base of the mat or associated with crop residues, and in the ground where it moves by walking (25 m in 6 months) and rarely on the fly (Gold and Messiaen, 2000). *Cosmopolites sordidus* prefers places with high humidity where they settle by the phenomena of contact, chemo-reception, thigmotactism, and hygrotropism (Pavis, 1988; Musanza *et al*., 2003). Adults have essentially nocturnal activity, with negative phototropism. *Cosmopolites sordidus* can withstand 3 to 10 days on the dry substrate (Gold *et al*., 1999); but on the wet substrate, it can survive up to 7 months without food (Musanza *et al*., 2003).

Cosmopolites sordidus obeys the "K" selection mechanism with a longevity of 1 to 4 years (Gold and Messiaen, 2000). It is a holometabolan insect with completed metamorphose. Their development cycle is adult maturation, reproduction, and eggs laying, development of larva, pupa, and adults of the new generation. Young adults of *C. sordidus* are brown (fig. 5), they turn black (fig. 5) after at least 20 days depending on temperature; they measure between 10 - 15 mm at the end of their development cycle (personal observation; Gold and Messiaen, 2000). The recognition of sex is done by the width of the individual, the punctuation, and the color of the rostrum, the abdomen, and by other secondary sexual characters. For example, females are large (Gold *et al*., 1999), with a reddish-accented rostrum while males are formed of the abdomen with oscillating segments (lateral view) (Musanza *et al*., 2003). *Cosmopolites sordidus* breeds only by sexual reproduction; males and females are sexually mature respectively 18 days and 5 days after emergence at room temperature (Uzakah *et al*., 2017). They produce both sexual aggregation pheromone which favors attraction between males and females (Budenberg et *al*.,1993; Uzakah *et al*., 2015). The inseminated female (during copulation) dig holes in the bulb of the banana tree using their rostrum and lays elongate (1 - 2 mm) and oval cream-white eggs (Gold and Messiaen, 2000; Uzakah *et al*., 2017) (fig. 5). The banana weevil has a low fecundity with an estimated laying rate of 1 to 3 eggs per week. These eggs hatch and give creamy white larvae, with streaks in the abdomen and a reddish head. The larvae pass through 5 to 8 larval stages and then pupate as white nymphs (12 mm long) that later emerge as young brown adults (Gold and Messiaen, 2000) (fig. 5). Like all insects, *C. sordidus* are poikilotherm, meaning that temperature affects all the aspects of their life: development rate and time, emergence, and oviposition. Laboratory studies showed egg hatching takes about 4-7 days at 23 - 25 ºC (Gold *et al*., 1999), 6 to 8 days under tropical

conditions (Treverrow, 2003), 4 - 15 days at 25 – 28 ºC (Musanza *et al.*, 2003); the larval stage lasts 25 - 32 days between 20 – 25 ºC and 23 days between 25 – 28 ºC(Musanza *et al.*, 2003).The development time from the nymphal stage to the adult stage is 7 to 10 days.

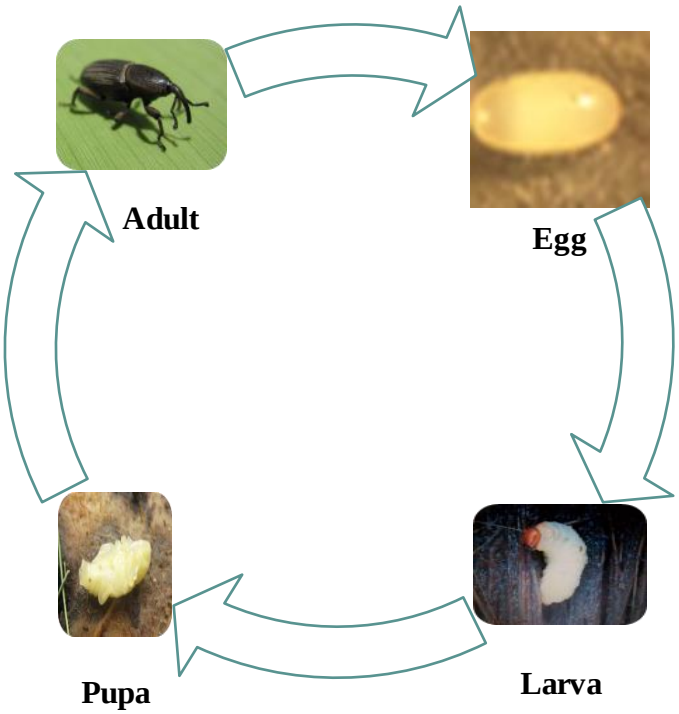

Fig. 5. Life cycle of banana borer, *C. sordidus* (modified Debonnaire, 2011)

I.2.4. *Cosmopolites sordidus* damage to banana

Of the 4-species identified in Cameroon, *C. sordidus* is the most economically important in banana and plantain (Ysenbrandt *et al.*, 2000, Okolle *et al.*, 2009). The larval stage is the most destructive (Musanza *et al.*, 2003; Sadik *et al.*, 2010). Larva feeds on the oviposition site and digs tunnels that can reach up to about 8 mm in diameter (Sadik *et al.*, 2010).

Larval activities can interfere with root initiation and sap uptake in the plant; as a result, the leaves turn yellow, wither, and die prematurely starting from the youngest leaves (Treverrow, 2003). Young suckers show symptoms of wilting and die, but older plants are retarded in their growth (Akollo *et al.*, 2008, Armendariz *et al.*, 2016). Heavily infestation can cause premature young plant death (75 %), reduction of bunches size, plants falling, and

reduction of plantation lifespan (Ysenbrandt *et al.*, 2000). The banana weevil damage is higher in low altitude areas compare to highland areas because of the influence of temperature (Erima *et al.*, 2017). The damages vary across agro-ecological zones, production systems, and cultivars. It is known that Musa AAB is more susceptible to *C. sordidus* compare to bananas (Ysenbrandt *et al.*, 2000). An infected plant is characterized by external and internal symptoms such as yellowing of the leaves (fig. 6A), the presence of dark galleries in the bulb and pseudo-stem (fig. 6B), premature death of young plants (Gold and Messiaen, 2000). External symptoms are often confused with manifestations due to stress (water or poor nutrition).

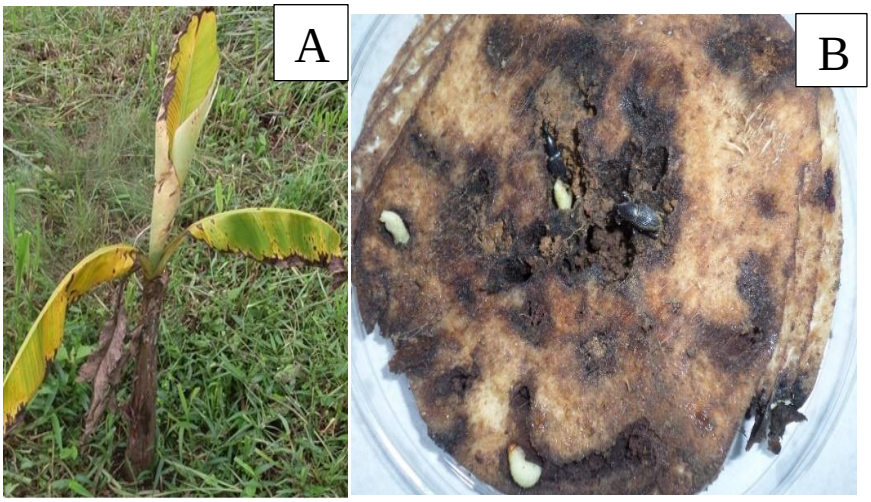

Fig.6. External and internal symptoms due *C. sordidus*: A: infested plant; B: Bulb presenting galleries excavated by the larvae.

I.2.5. Management of banana weevils

Control methods against *C. sordidius* are cultural, chemical, genetic, and biological (Okolle *et al.*, 2009).

I.2.5.1. Cultural control

Several cultural practices are used in the banana farming system for the management of *C. sordidus* (Sivirihauma *et al.*, 2017). Banana weevil management using cultural strategies are focus on manipulation of the crop or crop environment to prevent and limit the establishment of weevil populations. It is recommended to use the healthy plant material (Gold

et al., 2003) resulting from the micro-propagation and the macro-propagation or make the trimming of suckers associated with the treatment in hot water at50 to 55 ºC for 15 to 25 minutes. Cultural practices also include deep planting, weeding, mulching, application of organic manure or fertilizer, control of plant residues known as oviposition site (Gold *et al.* 1999), and systematic trapping with pseudo-stem or rhizome pieces, as well as the removal of crop residues (Musanza *et al.*, 2003). Intercropping systems, fallowing, soil cover and recession of remnants, and crop rotation with tolerant or resistant crops of *C. sordidus* also reduce the parasite pressure (Masanza,2003; De Graaf *et al.*, 2007; Sivirihauma *et al.*, 2017). Crops such as coffee (Masanza, 2003), potatoes, and pineapples can be planted before banana re-culture (Musanza *et al.*, 2003; Okolle *et al.*, 2009).

I.2.5.2. Genetic control

Genetic methods consist of selecting resistant and tolerant varieties, make crosses to produce weevil-resistant hybrids and incorporation of genes from various sources (Kiggundu et *al.*, 2003b; Okolle *et al.*, 2009). Considerable works have been done in screening diverse *Musa* germplasm for weevil resistance in Africa and Asia (Fogain and Price 1994; Pavis and Lemaire, 1997; Kiggundu *et al.*, 2003). Although known as the most susceptible, some plantain cultivars were found resistant to the banana weevil: Karumpoovan and Poozhachendu in India (Padmanaban *et al.*, 2001); Kedong Kekang in Cameroon (Fogain and Price, 1994). Intermediate resistance to weevils was obtained with some highland banana cultivars: Tereza, Nalukira, and Nsowe (Kiggundu *et al.*, 2003). Some cultivars of the AAA genome group were found to be more resistant to *C. sordidus* (Musanza *et al.*, 2003). Following genetic varietal selections, cultivars such as FHIA03, Yangambi Km5, and IITA hybrids: TMBx612-74, TMB2 × 8075-7; TMB2 × 7197-2; TMB2 × 6142-1 were found resistant to banana weevil (Kiggundu et *al.*, 2003b; Arinaitwe *et al.*, 2014).

I.2.5.3. Chemical control

Chemical control is one of the old strategies used in pest management. Chemical products are applied variously: directly against adults (Collins *et al.*, 1991); by dipping corms in insecticide solution (Cardenas Murillo *et al.*, 1986), by introducing the pesticide in planting holes or on trap (Fogain *et al.*, 2002; Fancelli *et al.*, 2013), and at the base of the banana mat

(Fogain *et al.*, 2002). The use of chemical insecticides helps keep the pest population below the rate responsible for significant economic damage but can also cause detrimental effects on beneficial insects (Collins et *al.*, 1991; Masanza *et al.*, 2003). Organochlorine, organophosphate, and carbamate insecticides have been used against the banana borer. Some effective synthetical pesticides such as DDT, chlordecone, and Dieldrin have caused pollution of agricultural lands and groundwater and even caused resistance to the weevils (Nankinga, 1994; Mitchell, 1980). In Cameroon, many insecticides have been recommended against banana weevil but Regent® and Counter® granules were found more effective (Mongyeh *et al.*, 2015).

I.2.5.4. Biological control

Biological control is an alternative of pest control using naturally occurring substances or organism that controls pests in an eco-friendly manner. Natural enemies such as predators, parasitoids, and pathogens have been used to reduce the population density of *C. sordidus* to lower the level of economic damage and bring the population back to a tolerable equilibrium. Some insects in the orders Hemiptera, Dermaptera, Diptera, Coleoptera, and Hymenoptera are enemies of *C. sordidus* (Masanza, 2003). *Plaesius javanus* Richson, *Hyposolenus laevigatus* (Mar.), *Hololeptas quandridentata* (F.) (Histeridae), *Belolnuchius ferrugatus* Erichsoon, *Leptochirus unicolor* Lepeletier (Staphylinidae), *Cathartus* sp. (Silvanidae), *Dactylosterbum hydrophilordes* sp., *D. abdominale* F. (Hydrophilidae) and dipteran, *Chrysopilus ferruginosus* Wied are also natural enemies of *C. sordidus* (Cuille, 1950).

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are known as natural enemies of the various pest. They live in a mutualistic association with bacteria of the genera *Xenorhabdus* and *Photorhabdus* (Boemare *et al.*, 1993) respectively. During the infection process by the nematodes, the bacteria are released into the insect hemocoel where they multiply and cause septicemia resulting in the death within 48 h (Ndiritu *et al.*, 2016). The entomopathogenic nematodes *Steinemema carpocapsae*, *S. karii*, *S. anomali*, *S. yirgalemense*, *Heterorhabditis indica*, *H. amazonensis*, and *H. bacteriophora* were found pathogenic to *C. sordidus* larvae (Treverrow *et al.*, 1991; Bortoluzzi *et al.*, 2013; Ndiritu *et al.*, 2016). In addition to entomopathogenic nematodes, parasitoids, bacteria, endophytic or mycorrhizal fungi, and entomopathogenic fungi are used in the control of *C.*

sordidus and other coleopterans (Kaaya *et al.*, 1993; Ambang *et al.*, 2002; Akello *et al.*, 2008; Okolle *et al.*, 2009).

The potential of entomopathogenic fungi has been exploited as an active ingredient in the development of microbial biopesticide products. Among the entomopathogenic fungi studied, *Beaveria bassiana* (Bals.) Vuillemin and *Metarhizium anisopliae* (Metch) are microbial agents that shown great potential against adult weevils (Aby *et al.*, 2010; Lopes *et al.*, 2011; Omokoko *et al.*, 2014). In the Caribbean, *M. anisopliae* was effective in reducing the population of *C. sordidus* (by 50 %), bulb damage (60 %) with a 20 % yield increase (Masanza, 2003). Their performance in the field was also evaluated **(**Magara et *al.*, 2003; Moreira *et al.*, 2017; Fancelli *et al.*, 2013; Tinzaara *et al.*, 2015; Gonzalez *et al.*, 2018). The first report of entomopathogenic fungi presence in Cameroon was in 1994 (Fogain, 1994). *Beauveria bassiana* was found on the cuticle of *C. sordidus*. The strain caused 92 % mortality after 9 days (Fogain, 1994). Investigations on the potential of *B. bassiana* to control *C. sordidus* were also carried out in Cameroonon 40 indigenous isolates of *M. anisopliae* and *B. bassiana* which caused mortality of up to 96 % against adult *C. sordidus* (Membang, 2013).

I.3. Biopesticides as a pest management tool

I.3.1. Biopesticides market

Innovative technology has been developed in such a way that year after year the number of available and commercialized products is increasing (Akmal *et al.*, 2013). The safety, regulation function, and potential against insects, future market trade, availability in nature, and demand for alternatives to pesticides (Nicolopoulou-Stamati *et al.*, 2016) have increased interest in these natural resources. The estimation of the total market share of biopesticides is dependent on what is included as biopesticides. Most estimations are based on a restricted definition, for example, excluding genetically modified plants and augmentative natural enemies. The global market for biopesticides in terms of revenues is increasing progressively. Biopesticide sales in 2010 were estimated at 4.5 % of the world's pesticide market (Bailey *et al.*, 2010). Its outcome was estimated to approximately $3 billion in 2015 (Olson, 2015). In 2017, the biopesticides market was estimated at $3.22 billion and projected to reach 4.1 $ billion in 2018 and $6.60 billion by 2022, at a CAGR of 15.43 % (Markets and markets, 2018a). Its growth in the crop protection market can be attributed to

the rise in environmental concerns and awareness regarding hazards caused by chemical pesticides. Ease of application, innovative product offerings, increased availability, and growth in demand for organic food products are factors that further drive the growth of this market. Geographically, North America was the largest market for biopesticide in 2017, followed by Europe, Latin America, Asia, and the rest of the world. Many pesticide companies have shown interest in biopesticides by including biopesticides in their list of products (Beer, 2013). The global market for biopesticides is dominated by biopesticides manufacturing companies such as BASF (Germany), Bayer (Germany), Monsanto BioAg (US), BioWorks (US), and Certis (US). Marrone Bio Innovations (US), Koppert (Netherlands), Arysta LifeScience (US), Valent BioSciences (US), Certis (US), Stockton Group (Israel), Isagro (Italy), and Camson Bio Technologies (India) are a few other key market players that also have a significant share in the biopesticides market. Raw material suppliers, Research and Development institutes and government bodies, and regulatory associations such as the US Department of Agriculture (USDA) are also involved in the value chain of the biopesticides market.

I.3.2. Biopesticides advantages and limitations

Pesticide use has certainly contributed towards improving agricultural production, in terms of both yield and quality, thus increasing agricultural income. However, the careless use of pesticides without adhering to the safety norms and recommended practices has posed serious health risks to humans, biodiversity, and the environment (Lenghai and Muthomi, 2018). The public awareness about the adverse effects of pesticides on the safety of foods and the environment has increase demand for food safety and quality; and have resulted in the search for safe and eco-friendly alternatives, renewed interest in the development and use of biopesticides in pest management products including biopesticides (Nollet, 2015; Nicolopoulou-Stamati *et al.*, 2016). Biopesticides are important tools in sustainable agricultural production since they are as effective as synthetic pesticides in the management of crop pests (Lenghai and Muthomi, 2018). Natural products are ecofriendly and easily biodegradable (Lenghai and Muthomi, 2018). Biopesticides have very short pre-harvest intervals and are therefore safe to use on fresh fruits and vegetables (Khater, 2012). They are also host-specific and hence do not affect the beneficial organisms or biodiversity (Lenghai

and Muthomi, 2018). Natural pesticides have a complex mode of action and no evidence of pest resistance has been shown (Lenghai and Muthomi, 2018). Some microbial pesticides based in *B. bassiana* have shown transmission potential in the host population (Lopes *et al*., 2011). The availability of biopesticides' active ingredients within the natural environment makes them inexpensive (Srijita, 2015). Biopesticides are safe products both for the applicant and the consumer and they can suitably be incorporated in integrated pest management (IPM) since they help to reduce the amounts of chemical pesticides used in the management of crop pests (Lenghai and Muthomi, 2018). Some biopesticides are also used in the decontamination of agricultural soils through the introduction of important microbial species (Javaid *et al*., 2016).

Biopesticides as a natural product, though provide such advantages as a safe environment and healthy food for human consumption, are affected by environmental factors that influence their efficacy and adaptability and hence consequently their full adoption (Maina *et al*., 2018). Their efficiency in the fields sometimes required high doses which makes them cost-effective. The lack of a readily available market makes it hard to invest in biopesticides (Lenghai and Muthomi, 2018). Some growers are reluctant to be advised on new systems and want to compare the effectiveness of biopesticides to traditional chemicals while they are measured differently. It is also clear that any single biochemical control method can rarely replace chemical insecticide treatments. (Nollet, 2015). Other perceived negative factors include expense (cost-effective), low efficacy, slow to kill, limited shelf life, high selectivity, and variability in performance (Nollet, 2015). These issues are sometimes more perceived than actual because no issue is universal to all biopesticides. There is also an issue that many biopesticides target only niche markets due to the specificity of the bioactive agent, which results in registration costs being too high for the size of the market, limiting commercialization possibilities. Registration of the products requires data on chemistry, toxicity, packaging, and formulation which is not always readily available (Gupta and Dikshit, 2010). The cost of producing a new pesticide product is usually high and has a lot of resource limitations (Lenghai and Muthomi, 2018).

I.3.3. Types of biopesticides

Biopesticides are a specific group of crop protection tools used in Integrated pest management (IPM). According to the Environmental Protection Agency (EPA) biopesticides are defined as "certain types of pesticides derived from natural materials such as animals, plants, microorganisms, and certain minerals". In the broadest sense, a biopesticide is simply a formulation based on the activity of an agent of natural origin that has pesticidal action (Glare, 2015). The term "biopesticides" has been used to cover a wide variety of formulated products that are used for the control of pests, diseases, and weeds. Chandler et *al* (2011) group biopesticides in three categories: semiochemical (pheromones); biochemical or botanical (plant-derived compound insect pheromone, plant extracts, and oils, plant growth regulators, and insect growth regulators) and microbial biopesticides (nematodes, bacteria, fungi, protozoa, and virus). Leng *et al* (2011) listed 4 categories: microbial pesticides, botanical biopesticides, zooid pesticides (animal toxin, insect hormone, pheromone and natural enemy as predators, parasites, etc.), and genetically modified plants while Nawaz *et al* (2016) listed three categories: microbial biopesticides (microorganisms), botanical biopesticides (plant-derived) and semiochemical. Some authors include beneficial predators and parasitoids in the group of biopesticides, while most do not (Lenghai and Muthomi, 2018; Chandler *et al.*, 2011). Although there is controversy in the classification of biopesticides, the majority of authors agree to list microorganisms or microbial agents as biopesticides (Chandler *et al.*, 2011; Leng *et al.*, 2011; Nawaz *et al.*, 2016; Lenghai and Muthomi, 2018).

I.3.4. Use of entomopathogenic fungi as microbial control agents

Entomopathogenic fungi are heterotrophic, unicellular, or filamentous eukaryotic micro-organisms that reproduce either sexually or asexually from spores, blastospores, microsclerotia, or conidia (Humber, 2012). These microorganisms play an important role in the natural regulation of insect populations (De Kouassi, 2001). There are about 700 to 1000 species of fungi that infect agricultural and forest insects (St Leger and Wang, 2010). They belong to the class of Chytridiomycetes, Oomycetes, Zygomycetes, Ascomycetes, and Deuteromycetes (De Kouassi, 2001). The greatest number of insect pathogenic fungi are found in the division of Zygomycetes, while the most used in biological control belong to the division of Deuteromycetes, especially the division of Hyphomycetes (De Kouassi, 2001).

Hyphomycetes include filamentous fungi formed by budding of the mycelium at the fertile end from the conidium. The well-known genera, Beauveria, Culicinomyces, Hirsutella, Metarhizium, Nomuraea, Paecilomyces, Tolypocladium, and Verticillium (Lecancilium) are generally used for the development of mycoinsecticides because of their high pathogenic potential. According to Faria et Wraight (2007), about 171 products based on entomopathogenic fungi are commercialized: *Beauveria bassiana* ((34 %), *Metarhizium anisopliae* (34 %), *Isaria fumosorosea* Wize (6 %) and *B. brongniartii* (Saccardo) Petch (4 %). Those products are commercialized under names such as BotaniGard® (*B. bassiana*), BioCeres® G WP and G WB (*B. bassiana*), Met 52® (Metarhizium), Boveril (*B. bassiana*), Metaril (Metarhizium), Vertalec (*L. longisporum*), Green-muscle, etc (Moreira *et al.*, 2017; Maina *et al*., 2018).

I.3.5. Case of Beauveria and Metarhizium

I.3.5.1. History and morphology of Beauveria and Metarhizium

Bassi (1835) in his studies of infectious diseases of the silkworm demonstrated that the fungus later described as *Botrytis bassiana* by Jean Beauverie (1911), was the causative agent of silkworm muscardine disease. It was until 1912 that Vuillemin gave it the name *Beauveria bassiana*. Currently, many other species can be distinguished such as *B. alba*, *B. amorpha* (von Hohnel, Samson and Evans), *B. brongniartii*, *B. velata*, *B. caledonica* (Bisset and Widden), *B. vermiconia* (Hoog and Rao), and *B. bassiana* (Vuillemin) based on morphological studies, biochemical and enzymatic tests (De Kouassi, 2001). Pasteur and Le Conte suggested using fungi to control insects in 1874. The first fungus to be used to control insects was *Metarhizium anisopliae*, formerly known as *Entomopthora anisopliae*. It was first discovered by Metchnikoff (1878) in studies of the diseases of wheat insects *Anisopliae austrica* and described by Sorokin (1883). In addition to *M. anisopliae*, there are other species such as *M. album*, *M. bruenneum* Petch (1935), *M. flavoviride* Gams, and Rozypal (1973), etc.

Beauveria bassiana and *M. anisopliae* develop as a slow-growing white cottony layer that turns yellow and green respectively (Humber, 2012). *Beauveria bassiana* is characterized by a filiform and serrated mycelium formed of long transparent septal zig-zag filaments whose diameter varies between 2.5 and 25 µm. The conidiogenous cluster-shaped apparatus is dense

and contains haploid and hydrophobic inflated cells called conidia. These conidia are produced on conidiophores in an aerobic environment, whereas in the absence of air oval blastospores (2 to 3 µm in diameter and 7 µm in length) are formed. *Beauveria bassiana* has hyaline conidia, globose, or subglobose (fig.7C) measuring 1.5 µm to 3.5 µm in diameter (Humber, 2012). *Metarhizium anisopliae* is distinguished by compact and scattered conidiophores that form dense clumps intertwined with whorled branches. Phialides are cylindrical and clavate 9 to 14 µm long, consisting of conidiogenous cells arranged in dense hymenium. Ovoid or cylindrical olive-colored conidia (fig.7F) are generally in a chain or parallel chain mass (Humber, 2012).

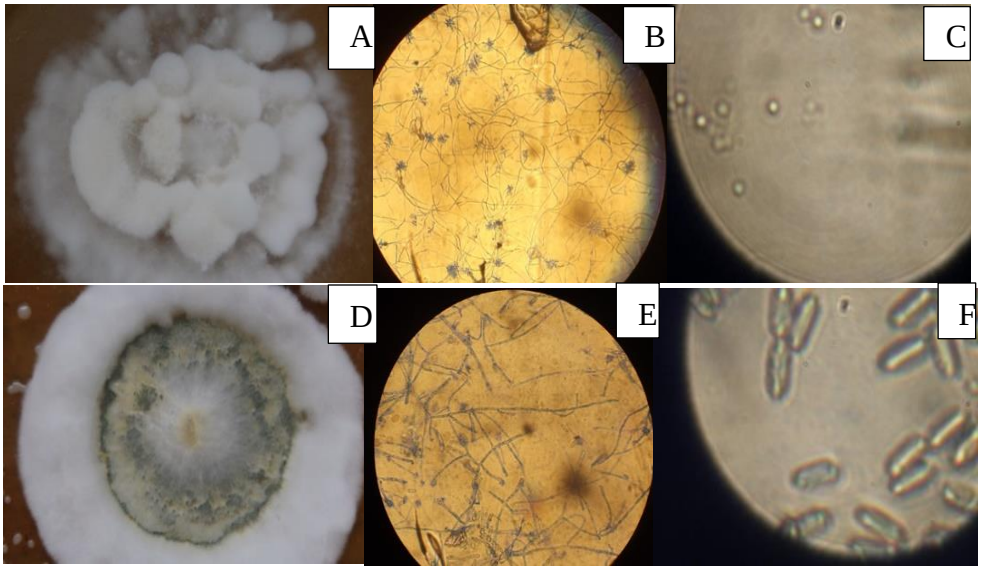

Fig. 7. Morphology of entomopathogenic fungi: Beauveria: A) colony B) mycelium and C) - Conidia; and Metarhizium D) colony E) mycelium and F) Conidia.

I.3.5.2. Bioecology and spectrum of host

Entomopathogenic fungi are cosmopolitan, meaning that they can be found in many habitats: aquatic environments, forests, agricultural areas, pastures, desert areas, and even urban habitats (Meyling and Eilenberg, 2007; Vega *et al.*, 2012). According to Meyling and Eilenberg (2007), the soil is the conventional medium for the isolation of entomopathogenic fungi. It remains the best habitat and refuge, able to protect *B. bassiana* and *M. anisopliae* against ultraviolet radiation and other biotic and abiotic factors (Meyling and Eilenberg,

2007). Studies on *M. anisopliae* revealed that they are abundant in cultivated soils and forests (Bidochka *et al.*, 1998; Meyling and Eilenberg, 2007). *B. bassiana* is saprophyte in both cultivated and non-cultivated soil and its ecology often dependent on its role. It is a preferential species of humid soils (tropical and subtropical) characterized by high humidity, the temperature is around 20 - 30 ºC, and pH value 5 - 7 (Taylor and Khan, 2010; Mishra *et al.*, 2013).

Recent studies have shown that *B. bassiana* and *M. anisopliae* can interact with plants. *B. bassiana* as endophytic fungi, has a high spectrum of host plants including maize, potato, cotton, tomato, opium, coffee, cocoa bananaand many others (Akello *et al.*, 2007; Parsa *et al.*, 2013). The fungi (*B. bassiana*)-plants interaction is a mutualistic relationship in which the endophytic fungus by its presence in plant tissues protects against herbivorous insects (Meyling and Eilenberg, 2007).

Beauveria bassiana and *M. anisopliae* are also known as natural enemies of many arthropods and arachnids (Meyling and Eilenberg, 2007). They interact with the insect and cause white or green muscardine. *Helicoverpa armigera, Alphitobus diaperinus, Plutella xylostella, Laniifera cyclades, Bemisia tabaci* (Taylor and Khan, 2010), *Galleria mellonela, Tenebrio molitor* (Meyling and Eilenberg, 2007), *Aphis gossypii, Spoladea recurvalis* (Opisa *et al.*, 2019), *Aphis craccivora* (Mweke et *al.*, 2018) and *C. sordidus* (Tinzaara et *al.*, 2015; Moreira et *al.*, 2017; Gonzalez *et al.*, 2018) are a non-exhaustive list of host insects of *B. bassiana* and *M. anisopliae*. Entomopathogenic fungi can persist in the environment as conidia (Vannienen, 2000).

I.3.5.3. Life cycle of *Beauveria bassiana* and *Metarhizium anisopliae* on an insect

The fungi Beauveria and Metarhizium infect insects by direct contact of conidia on the insect cuticle, through the application or horizontal transmission (Godonou *et al.*, 2000; Lopes *et al.*, 2011; Opisa *et al.*, 2019). The infection process starts from the cuticle, goes through the epidermis, and finally gets into the hemocoel (fig.8).

The mode of infection can be subdivided into several phases: the adhesion, germination, the penetration of hyphae, differentiation (or internal proliferation by blastospore formation *in vivo*), and the formation of muscardine.

I.3.5.3.1. Adhesion and germination

Infection begins with the attachment of fungus propagules (conidia or blastospores) on the insect cuticle. The hydrophobic and electrostatic forces of conidia favor the attachment of conidia to the cuticle (De Kouassi, 2001). Ortiz-Urquiza and Keyhani (2013) reported that adhesion could be achieved in three consecutive steps: the adsorption of conidia on the surface of the cuticle, the adhesion of the interface between the conidia and the epicuticle, the conidia germination and hyphae growth on the surface of the insect cuticle (fig. 8).

Conidia adhere to the surface of the insect cuticle through hydrophobic and electrostatic charges (Boucias *et al.*, 1988; Butt *et al.*, 2016). Some adhesive proteins (MAD1 produced by *M. anisoplaie*) or hydrophobin (produced by *B. bassiana*) are involved in the adhesion during the recognition mechanism (Butt *et al.*, 2016)Adhesion of conidia is possible when the recognition mechanism and compatibility between the conidia and the integumentary cells of the insect are positive (De Kouassi, 2001). The adhesion phase results in the germination of conidia. Expression of various hydrolytic enzymes (e.g: proteases, chitinases, and lipases) and other factors (such as temperature, humidity, nutrients, and physicochemical factors of the host (Butt *et al.*, 2016)), promote germination and growth of the fungus across the surface of the host, and subsequent penetration of cuticular layers. During this process, the fungus produces any number of specialized infection structures that can include penetration pegs and/or appressoria, which enable the growing hyphae to penetrate the host integument. Besides, certain enzymes, such as endoproteases and aminopeptidases, are also produced during conidial germination.

I.3.5.3.2. Penetration of the hyphae through the cuticle of the insect

Entomopathogenic fungi penetrate insects by crossing cuticle layers of the insect (epicuticle, procuticle, and epidermis) and reaching hemolymph which contains nutrients necessary for fungal development. The penetration sites are observed as dark lesions in the epicuticle indicative of enzymatic action and mechanical pressure (Shahid *et al.*, 2012). Extracellular enzymes such as proteases, lipases, and chitinases are produced by hyphae for degradation of insect's cuticle (Butt *et al.*, 2016). *B. bassiana* can secrete Pr1 and Pr2 proteins (Butt *et al.*, 2016). Chitinase is also involved in hyphae formation and conidiogenesis (Butt *et al.*, 2016).

I.3.5.3.3. Muscaridine formation

After penetration, hyphae reach the hemocoel and produce hyphal bodies namely blastospores that circulate through the hemolymph (Tanada and Kaya, 1993) and multiply by budding. These blastospores have unicellular structures where their cell membrane is replaced by a thin layer above the plasma membrane (fig.8). When temperature and humidity conditions are met, 3 to 10 days after infection, the insect dies, and the fungus produces an antibiotic called oosporin to resist bacteria in the intestinal tract of the insect. Mycotoxins are also produced to cope with the insect's immune system (Tanada and Kaya, 1993). Beauveria , for example, produces insecticidal toxins such as beauvericin, enniatine, and bassianolide while *M. anisopliae* produces metalloproteases and chitinolytic enzymes.

After the death of the insect, a white cottony layer develops on the insect. During sporulation, this white layer becomes green or yellow respectively for *M. anisopliae* and *B. bassiana*.

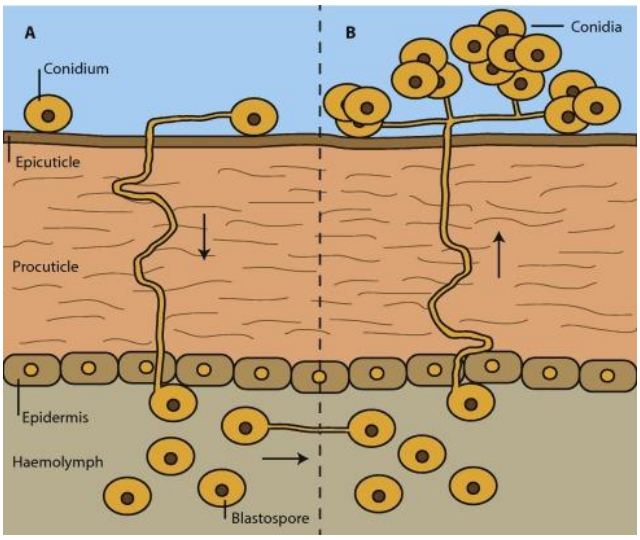

Fig. 8. Mode of action for entomopathogenic fungi: A) and B) saprophytic phase

I.3.6. Factors affecting the efficacy of entomopathogenic fungi

I.3.6.1. Host and pathogen factors

The susceptibility of insects to entomopathogenic fungi is influenced by factors including morphology, host physiology, and environment (Inglis *et al.*, 2001). As far as interaction fungal-host is concerned, the effectiveness of an isolate can vary with the life

stages of insects due to the composition of the cuticle (Ortiz-Urquiza and Keyhani, 2013). Ansari and Butt (2012) reported that larvae and pupae of *Hylobius abielis* (Coleoptera: Curculionidae) were highly susceptible to entomopathogenic fungi pathogens compare to adults. The defense system of an insect can also influence the efficacy of entomopathogenic fungi, for example, some insect can exude glandular secretions which display antifungal activity against entomopathogenic fungi at certain life stages (Ortiz-Urquiza and Keyhani, 2013). Some entomopathogenic fungi as Beauveria and Metarhizium are known to disseminate through contact between an infected and healthy insect (horizontal transmission). The transmission potential of a fungus strain is related to the behavior and density of insects. However, it can also increase the virulence of a fungus (Membang *et al.*, 2020). Some social insects act strangely by repellency towards conidia, by allogrooming to remove conidia from infected insect, by alarm behavior such as walking off of infected colonies, avoiding or burying infected cadavers response (Yanagawa *et al.*, 2007; PikKheng *et al.*, 2009; Yanagawa *et al.*, 2012). They can also use the immune response by producing antimicrobial compounds, and the activation of a cellular and humoral response (Aw and Hue, 2017**).** These insects may have sensorial receptors that signal the presence of a microbial agent in their environment. All steps of the infection process are determinant for pathogenicity (germination speed, hyphal growth, the formation of blastospores, and muscardine formation).

I.3.6.2. Environmental factors

Environmental factors have been highlighted as a principal cause of the reduction of entomopathogenic fungi performance in the natural environment (Maina *et al.*, 2018). The efficacy of entomopathogenic fungi is influenced by environmental factors including solar radiations, temperature and relative humidity.

I.3.6.2.1. Solar radiation

The solar spectrum contains electromagnetic radiation at different wavelengths. Ultraviolet (UV) radiation particularly can inactivate conidia and cause lethal damage to DNA and mutations which consequently reduces fungal efficiency in the field (Braga *et al.*, 2001b). Conidia and hyphae of entomopathogenic fungi are very sensitive to UVB of spectrum 285 - 315 nm. The exposure of fungi to UVB light may delay conidial survivorship and reduce

fungal development, resulting in decreases in the persistence and efficacy of infective propagules in the field (Zimmermann, 1982; Roberts and Campbell, 1977). The sensitivity of entomopathogenic fungi varies between fungal species and strains (Fargues *et al.*, 1996). Other studies controversially revealed that exposure of fungi mycelium to UV radiation can stimulate the activity of entomopathogenic fungi (Zhang *et al.*, 2009; Sahab *et al.*, 2014).

I.3.6.2.2. Temperature

Temperature is known to be a critical and ubiquitous environmental signal that governs the development and virulence of diverse microbial species (Alali *et al.*, 2019). It affects germination, growth, and sporulation in fungal-host interaction. The thermal tolerance range depends on fungal adaptivity potential related to their genetic and origin. Borisade and Magan (2014) reported that some strains of *M. anisopliae* can grow at a temperature above 37 °C with the optimal range for germination, growth, and conidiation ranging between 20 and 30 ◦C. However, an infection can occur at temperatures ranging between 15 and 30 ◦C (Inglis *et al.*, 2001; Taylor and Khan, 2010; Mishra *et al.*, 2015). Microbial agent's tolerance depends on fungi species and strains since fungal can develop diverse molecular strategies to sense fluctuations in temperature, and nearly all cellular molecules, including proteins, lipids, RNA, and DNA can act as thermos-sensors (Shapiro and Cowen, 2012).

I.3.6.2.3. Relative humidity

Relative humidity has been considered for a longtime as a limiting factor for the use of entomopathogenic fungi as a tool for post-harvest insect management (Athanassiou *et al.*, 2017). Some strains of entomopathogenic fungi were found highly virulent against insects at high humidity (Fargues and Luz, 2000); whereas Lord (2007), Athanassiou *et al* (2017) proved that the infection process can occur independently of high humidity. In some cases, the humidity of the insect surface favors fungal growth (Fargues *et al.*, 1997b; Faria and Wraight, 2001). High humidity is required for germination and fungal growth for infection on insect cuticles (Zimmerman, 2007). The high conidia production of *Metarhizium anisopliae* on insect cadavers also depends on humidity (Athurs and Thomas, 2001; Fargues and Luz, 2000). Sandhu *et al* (1993) showed that low temperature and low humidity are desirable for long storage of dry conidia. The survival of conidia in the release environment also needs high

moisture (Zimmernann, 2007; Sandhu *et al.*, 2012). The combination of temperature and humidity also affects fungal development and effectiveness (Jaronski, 2010).

I.3.6.2.4. Soil characteristics

Soil characteristics (organic matter content, water saturation, microflora and microfauna antagonists, pH), the presence or absence of susceptible hosts for fungi, and chemical pesticides (Triademefon) applied in agriculture may also have a low or harmful to entomopathogenic fungi (Inglis *et al.*, 2001, Meyling and Eilenberg, 2007). However, pH of a treated surface can also influence conidia persistence or conidia survival and consequently pathogenicity (Inglis *et al.*, 2001). Many studies have been done on pH toxicity of various strains of Beauveria and Metarhizium, at different incubation times and medium types (Hallsworth and Magan, 1996; Kotwal *et al.*, 2012; Barra *et al.*, 2015; Otgonjargal *et al.*, 2015). The pH range for optimum germination, growth, and conidiation varied between isolates and it is surely related to genetic diversity (Padmavathi *et al.*, 2003). The entomopathogenic fungi can modify the pH of a medium (Raya-Diaz *et al.*, 2017). The tolerance and optimum ranges for germination, growth, and sporulation vary a lot (St Leger *et al.*, 1998; Raya-diaz *et al.*, 2017). *Beauveria bassiana* strains can tolerate a pH ranging between 2 and 14 (Padmavathi *et al.*, 2003; Otgonjargal *et al.*, 2015). Reports on pH toxicity on entomopathogenic fungi revelated that some strains had a specific pH for optimum germination which is pH 7.5 (Namasivayam *et al.*, 2015) or a narrow range (Padmavathi *et al.*, 2003; Mishra *et al.*, 2013) while others have a wide range (Padmavathi *et al.*, 2003).

I.3.7. Effect on non-target insects

Beauveria bassiana and *M. anisopliae* are known to have broad host ranges (Zimmermann, 1993). Their use as biological control agents usually relies on virulence on the target pest. However, the effect of fungus on beneficial insects and natural enemies of insect pests is also considered. Thungrabeab and Tongma (2007) reported that *B. bassiana* was found to be non-pathogenic to natural enemies and beneficial soil insects. *Metarhizium anisopliae*, instead, was pathogenic to *Chrysoperla carnea* and *Dicyphus tamaninii*. However, pathogenicity was low ranging between 4 to 10 %.

Danfa *et al.* (1999) showed that *Metarhizium* IM I 330 189, a potential biocontrol agent against locust and grasshoppers causes no infection on two non-target Sahelian tenebrionid beetles *Pimelia senegalensis* and *Trachyderma hispida*. However, exposure of the hymenopteran parasitoids *Bracon hebetor* (Braconidae) and *Apoanagyrus lopezi* (Encyrtidae) to surfaces treated with the same entomopathogens at a rate equivalent to ca. 5.3×10^{12} spores caused 100 % mortality.

In aquatic animals, no problems have been reported with *Metarhizium anisopliae* (Ganthner *et al.*, 1995). Donovan-Peluso *et al.* (1980) reported that exposing frogs to *M. anisopliae* spores by gastric intubation produced no pathologic lesions or infection of Viscera. However, a related insect pathogenic fungus, *Beauveria bassiana*, caused a fatal infection in a captive alligator (Fromtling *et al.*, 1979). Insect pathogenic fungi are safe for animals, plants, and the environment (Lacey, 2012; Vega and Kaya, 2012).

The type of application can also have affect non-target species. Inundative application of *B. bassiana* spores has, over time, shown no phytotoxic activity (Zimmermann, 2007). Also, for soil organisms such as springtails and moths, no significant adverse effects were noted following the presence of *B. bassiana* (Zimmermann, 2007). However, some adverse effects from its use on a few species of predators, parasitoids, and beneficial organisms such as pollinators have been observed. *Beauveria bassiana* reduces longevity and causes fungus at 10^6 - 10^8 spores/individual in the honeybee *Apis mellifera* L., in the laboratory (Vandenberg, 1990). Concerning humans, the few reported cases involve immuno-deficient individuals (Zimmermann, 2007). Indeed, it has been demonstrated that existing commercial formulations pose no risk to the health of humans and animals since the compounds secreted by the fungus are specific to the host it attacks, and the amount of inoculum produced in the environment is not abundant (Strasser *et al.*, 2000). As for *M. anisopliae*, according to Kabaluk *et al.* (2001), there is a high specificity of hosts depending on the strain. Although this limits its use as a general-purpose insecticide, it also limits the effects of the fungus on non-target organisms and makes it a safer product. Environmental Protection Agency expects no risk to humans when using products containing *B. bassiana* (EPA, 2011).

I.3.8. Biotechnology on entomopathogenic fungi based biopesticides

I.3.8.1. Mass production and preservation of spores

The mass production of insect-pathogenic fungi is a prerequisite for any large-scale field application and industrialization. Fungal spores are usually cultured in aseptic conditions (sterilized medium, air, and equipment) by a liquid, solid-state, or diphasic fermentation (Jaronski and Mascarin, 2017).

Liquid fermentation can be classified into two categories: submerged liquid fermentation and stationary liquid fermentation. The submerged fermentation is a fermentation where blastospores, microcycle conidia, or microsclerotia are formed when a fungus is submerged in a constantly agitated and aerated liquid medium. Contrary to submerged liquid fermentation, liquid media is kept without shaking during the incubation period then mycelium and aerial conidia are harvested for stationary liquid fermentation where sporulation takes place on a still and liquid surface (Jaronski and Mascarin, 2017). Liquid fermentation is done in media as potato dextrose broth and low glucose-medium (Jaronski and Mascarin, 2017).

Solid substrate fermentation is the production of aerial conidia by inoculating conidial suspension on a humid solid substrate. Biphasic fermentation is a fermentation where the subsidiary term a liquid fermentation inoculum is used rather than a conidial suspension to initiate the solid substrate phase. Several solid substrates have been used for mass production of entomopathogenic fungi including flours (cassava flour), grains or cereals (corn, barley, rice, millet, pea, wheat, and sorghum), cottonseed and residuals (vegetable waste, rice husk sugarcane bagasse, decomposed sweet potatoes, and mixtures) (Latifian *et al.*, 2013; Ibrahim *et al.*, 2015). The inert substrates such as cellophane and nutrient-impregnated membrane are also used as a base for the aerial growth and sporulation of Hypocreales fungi enables the complete separation of the pathogen from the nutritive medium (Jaronski and Mascarin, 2017. The quality control test is usually performed on the final product to ensure high conidia viability, free of potentially dangerous contaminants, virulence, moisture content (for long shelf-life), and the number of infective propagules per gram of product (Latifian *et al.*, 2013).

The spores obtained from mass production must be kept dormant, but alive, during storage. Fungal propagules for storage in dry conditions are generally harvested after air-dried for several days in a higher biosafety cabinet; separated using a vibratory sieve of vacuum

method (Jaronski and Mascarin, 2017) or myco-harvester; and then maintained over anhydrous silica gel crystals. Fungal propagules can be stored for a short or long time either by immersion in sterile distilled water, in mineral or vegetable oil, or by lyophilization or on silica gel at temperatures ranging from 4 ° C to -86 °C. The shelf-life of fungal propagules depends much on the species and/or strain, moisture content or relative humidity and storage temperature.

I.3.8.2. Formulations

Entomopathogenic fungi are usually used as an active ingredient in the development of biopesticide formulation for commercialization. All stages from the production of propagules to its eventual action on the target must be considered in formulation technology. Microbial agents are formulated for four good reasons: (1) to stabilize the organism during production, distribution, and storage; (2) to aid handling and application of the product so that it is easily delivered to the target in the most appropriate manner and form; (3) to protect the agent from harmful environmental factors at the target site, thereby increasing persistence and (4) to enhance the activity of the organism at the target site by increasing its activity, reproduction, contact, and interaction with the target pest or disease organism (Burges *et al*., 1998). The formulation process leads to a final product by mixing the microbial component with different carriers and adjuvants. There are two types of biopesticide formulation regarding their physical state: liquid and dry formulations. Liquid formulations can be water-based, oil-based, polymer-based, or combinations. Water-based formulations (suspension concentrate, suspo-emulsions, capsule suspension) require adding of inert ingredients, such as stabilizers, stickers, surfactants, coloring agents, antifreeze compounds, UV protectant, and additional nutrients. Dry formulations can be produced using different technologies, such as spray drying, freeze-drying, or air drying either with or without the use of a fluidized bed. They are produced by adding a binder, dispersant, wetting agents, UV protectant, etc. (Gašić and Tanović, 2013). Each formulation type is produced in a specific way. Dry formulations such as dust (DP), seed dressing formulations – powders for seed dressing (DS), granules (GR), micro granules (MG) are usually formulated for a direct application while others are formulated for dilution in water (water-dispersible granules (WG), and wettable powders (WP)). There are also liquid formulations for dilution in water (emulsions, suspension

concentrates (SC), oil dispersions (OD), suspo-emulsions (SE), capsule suspensions (CS), and ultra-low volume formulations (Gašić and Tanović, 2013). The choice of the type of formulation to develop depends on several factors, such as the type and location of the target, availability of formulation materials and application equipment, as well as user preference (Burges *et al.*, 1998). A single organism can be formulated in several different forms due to the climatic adaptability, targets, and user preferences according to the market (Burges *et al.*, 1998). The use of entomopathogenic fungi against soil insects is best accomplished with granular formulations. Banana weevil *C. sordidus* have been managed using dry and liquid formulations (dry powder, maize-based formulation, conidial suspended with dispersants (Nankinga *et al.*, 1999; Godonou *et al.*, 2000; Magara *et al.*, 2003; Fancelli *et al.*, 2013; Moreira *et al.*, 2017; Gonzalez *et al.*, 2018).

I.3.8.3. Delivery systems

The field application of a biopesticide requires an adequate system to spread and deposit the microbial agents. The choice of correct equipment is therefore essential to ensure that the biopesticide is applied effectively at the correct rate and on the target surface. It depends, among other things, on the size of the particles entering the compositions of the final products. Most equipment for biopesticide application has nozzle droplet sizes between 10 - 200 μm (Gan-Mor *et al.*, 2014). The application method depends on the type of formulation.

The solid formulations required a good applicator able to deliver accurately calibrated amounts of product and spray them evenly, without damaging products by grinding or impaction. The machine should be robust and easy to handle, calibrate, and repair, as well as inexpensive. Specialized equipment ranges from simple hand-held devices such as a 'pepper-pot shaker', which is particularly suited to small-plot, low-input agriculture and generally allows the product to be placed accurately on the target- to large tractor-borne equipment and aircraft suited to application over a large area (Burges *et al.*, 1998). Liquid formulations are applied using sprayers of various volumes. Microbial biopesticide is usually applied alone or in combination with other strategies. Beauveria and Metarhizium biopesticide were applied against *C. sordidus* alone or in combination with attractant, pheromone "cosmolure" (; Tinzaara *et al.*, 2005; Tinzaara *et al.*, 2011; Tinzaara *et al.*, 2015; Aby *et al.*, 2015).

CHAPTER II: MATERIAL AND METHODS

II.1. Study area

This work was carried out in the laboratory of pathology and entomology IITA-Cameroon located at Nkolbisson Yaounde. A Semi-field trial was done in the screening house and experimental fields of the same institution.

II.2. Material

II.2.1. Biological Material

II.2.1.1. Banana and plantain plants

Suckers of plantain cultivars "Assung-mbele" were collected from Ntui (Mbam et Kim division) and *vitro* plants of white Ebang produced by IITA's tissue culture laboratory were used for the assessment of biopesticide performance against banana weevil and endophyte test respectively. Banana and plantain suckers of cultivars Essong and William were collected monthly from a smallholder field at Awae for black weevil rearing. The choice of these cultivars was motivated by their economic importance, availability, and susceptibility to banana weevil (fig.9).

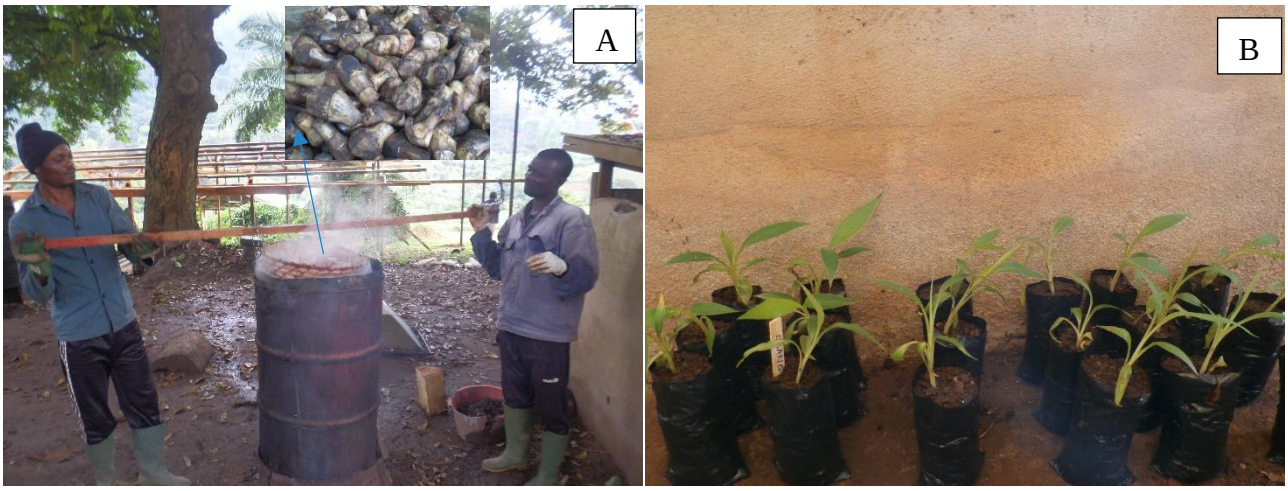

Fig. 9. Suckers treated with hot water (A) and vitroplant (B).

II.2.1.2. Entomopathogenic fungi isolates

The pure culture of *B. bassiana* BIITAC6.2.2; BIITAC10.3.3 and BIITAC8.1.5 and *Metarhizium anisopliae* MIITAC11.3.4; MIITAC6.4.2 and MIITAC6.2.2 provided by IITA fungi collection (Appendix 1) was used in this work (Membang, 2013). These fungi strains

were isolated from soil samples using a baited method (Meylind, 2007) with *C. sordidus* larvae. A previous study showed the virulence of the fungi against adult banana weevil (Membang, 2013).

II.2.1.3. Ingredients

Ingredients used in this study included: rice flour (C_1), gari powder (C_2), carrot flour (C_3), wood ashes (C_4), kaolin clay (C_5), baobab fruit powder (C_6), Mucuna leaves powder (C_7), charcoal powder (C_8), maize flour (C_9), cassava starch (C_{10}), milk powder (C_{11}) and banana flour (C_{12}).

II.2.1.4. Banana weevils

Black banana weevils, *Cosmopolites sordidus* (fig.10) were captured from IITA field using pseudo-stem traps and reared in the laboratory and screenhouse.

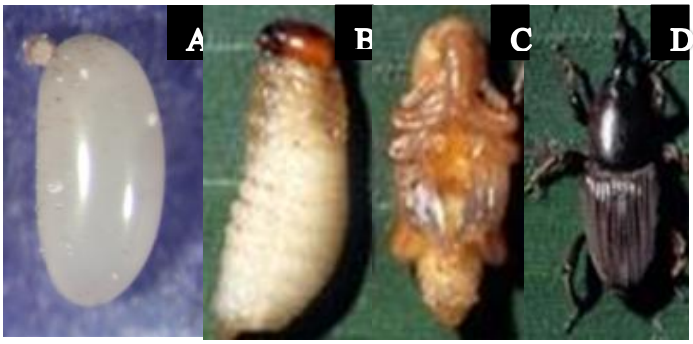

Fig. 10. Different life stages of *C. sordidus*: egg (A), larvae (B), pupae (C), and adult (D).

II.2.2. Technical Material

Petri dishes (55 mm and 99 mm diameter) were used for the culture of entomopathogenic fungi and parafilm to seal Petri dishes. Plastic bowls served as rearing containers for banana weevils' mass culturing. An agitator vortex permitted to homogenize the suspensions while pipettes and micropipettes were used to extract needed quantities of the suspension. Before using suspensions, they were kept in a refrigerator or a freezer at 4 ◦C to preserve the conidia from damages. All incubations were done in an incubation chamber (eggs, larva, adults, and culture media). Laminar flow cabinet, Watman paper, hydrophilic

cotton, and apparatus such as centrifuge, dryer, hobo, transilluminator, pH meter, hygrometer, etc were also used during our studies.

II.2.3. Synthetical Material

PDA powder was employed as a culturing medium to carry out germination tests, endophytic tests, and mycelia growth. Sulfate streptomycin was added as an antibiotic in culturing media to inhibit the growth of contaminants. Tween 80 was also used as a dispersant agent, sodium hypochlorite and alcohol 70 and 95 %, to disinfect hands and working surface.

II.3. Methodology

II.3.1. Characterization of fungal isolates

Characterization of entomopathogenic fungi strains was done based on two criteria: efficacy against *C. sordidus* life stages and endophytic potential.

II.3.1.1. Evaluation of efficacy fungal isolates against immature life stages of banana weevil

II.3.1.1.1. Collection and rearing of banana weevils

Banana root borer adults were collected from naturally infested banana fields at IITA-Cameroon (N03˚51′84", E11˚27′76") using pseudo-stem traps (Tinzaara *et al.*, 2011). These weevils were confined in an aerated plastic container (40 mm x 14 mm) with disinfected rhizomes of the local plantain variety Elat (AAB) as food (fig.11A). Containers were transferred in quarantine laboratory conditions of 25 ± 1 °C, 70 – 80 % relative humidity (RH), and total darkness for two weeks to confirm that the insects were not naturally infected with entomopathogens. Food was changed weekly before inspection of containers and dead insects were incubated on moist filter paper in Petri dishes for mycosis observation. None of the dead adult weevils showed any signs of mycosis. The living adult weevils after quarantine period were placed in containers with a trimmed plantain sucker (variety Essong, Asungbegle depending on availability) for weevil multiplication, following the method of Musabyimana *et al.* (2001) and Tinzaara *et al.* (2011), and to serve as a source of eggs and larvae to be used (fig. 11B, 11C and 11D) in subsequent experiments. Eggs led by second cohort adult BRB were harvested daily. The eggs were then transferred into a Petri dish lined with moist filter

paper (Night *et al.*, 2010) to conserve them in the incubation chamber at the conditions described above. Larvae obtained from hatched eggs were transferred daily into a plastic container and with crushed plantain corm as larval food. To obtain crushed plantain corm, the rhizome was trimmed, disinfected with hypochlorite 1%, and rinsed three times before mashing in a blender (Severin, KM 3881 made in Germany). Larval food was changed twice per week. One-day-old weevil eggs, 5th instar larvae, and pupae were harvested from the containers as needed for the various bioassays.

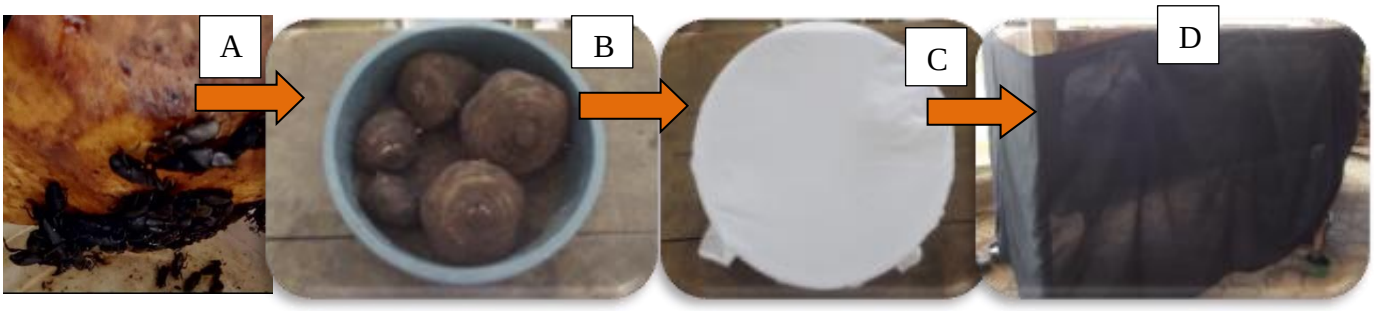

Fig. 11. Steps of banana weevil rearing in the screenhouse: A) infestation; B) infested corm; C) covering; D) incubation.

II.3.1.1.2. Entomopathogenic fungi cultures

The six isolates of entomopathogenic fungi used in this study were obtained from the IITA-Cameroon fungi collection: three *B. bassiana* isolates and three *M. anisopliae* isolates (appendix 1). All the isolates were obtained from soil samples collected in the Center region of Cameroon during various field surveys. Information on isolates collection is provided in appendix 1 (Membang 2013). The fungi were isolated with a baiting method (Meyling, 2007) and were identified based on Humber (2012) identification key (Membang, 2013). These isolates were found highly effective against *Sahlbergella singularis* Hagl. (Mahot *et al.*, 2019), *Nisotra uniformis* Jacoby (Niyibizi, 2018) and the adult stage of *C. sordidus* (Membang *et al.*, 2020). They were first re-isolated from adult weevils and cultured on potato dextrose agar (PDA) for 21 days at 25 ± 1 ˚C in dark and at 70 – 80 % RH. Fungal conidia of each isolate were scrapped and suspended in 10 ml Tween 80 solution. Conidia concentration for each fungus was then determined using a hemocytometer under a microscope at magnification

40X. The formula C1V1=C2V2 was used to dilute mother suspensions and obtain the needed dose, 3.2 x 10^8 conidia/ml.

II.3.1.1.3. Pathogenicity test

For the study of fungal isolates pathogenicity and virulence on immature stages - eggs of less than 24-hour-old, 5th-instar larvae, and 1-day-old pupae were artificially infected using the immersion method.

For bioassay on eggs, collected eggs were disinfected with 1 % hypochlorite and rinsed three times before immersing them in a conidial suspension. A batch of ten eggs was dipped for ~30 s in the fungal spore suspension of 3.2 x 10^8 conidia/ml (fig.12). Control eggs were also immersed similarly but in 0.1 % solution Tween 80. The egg bioassay consisted of 10 eggs in each treatment, replicated five times. The treated eggs were placed in Petri dishes with humid filter paper, sealed with parafilm, and incubated for 10 days at 25 ± 1˚C in darkness. After the incubation period, eggs and dead larvae were observed under a dissecting microscope to determine colonization of egg surface and infection of emerging larvae (Gindin et *al.*, 2006). Eggs mycosis, emerging larva mycosis, and combined mycosis rates were determined as follow:

- EMyc (%)= CE*100/ITE; where Emyc= egg mycosis; CE= number of surface colonized egg; ITE= number of initial treated eggs;
- ELMyc (%)= IL*100/NTL; where Emyc= egg mycosis; NCL= number of surface colonized neonate larvae; ITE= number of neonate larvae;
- CMyc (%)= (CE+IL)*100/ITE; where Cmyc= combined mycosis

Larvae and pupae were treated similarly. Larvae or pupa was disinfected in 1% sodium hypochlorite and rinsed three times using sterile distilled water. They were then immersed individually for ~30 s in 1 ml conidial suspension of 3.2 × 10^8 conidia/ml of each isolate, as described by Hasyim *et al.* (2009). Control insects were also dipped for ~30 s but in a solution of Tween 80 (0.1 % v/v), prepared with sterile distilled water. Fungus-treated and control insects were transferred to sterile cups containing mashed corm as food and incubated in a growth chamber at 25 ± 1˚C with constant darkness; and 70 – 80 % RH. Weevil mortality was monitored every 2 days for 14 days for larvae and 10 days for pupae. Each treatment consisted of five inoculated larvae or pupae per treatment, replicated five or six times respectively. Dead

weevils were disinfected as described above and incubated on humid filter paper for the fungal growth confirmation. Mortality and mycosis were recorded in both larvae and pupae bioassays. In addition to mortality and mycosis of emerged adult mycosis was monitored in the pupae bioassay (Gindin *et al.* 2006). Mortality and infection rates were determined as follow:

- LMort (%) = DL*100/ITL; where LMort = larval mortality; DL= number of dead larvae; ITL = number of initial treated larvae;
- LMyc (%) = CL*100/DL; where Lmyc = Larval mycosis; CL = number of surface colonized larvae; DL = number of dead larvae;
- PMort (%) = DP*100/ITP; where PMort = pupal mortality; DP= number of dead pupae; ITP = number of initial treated pupae;
- PMyc (%) = CP*100/DP; where Lmyc = Larval mycosis; CP = number of surface colonized pupae; DP = number of dead pupae;
- EAMort (%) = DEA*100/EA; where EAMort = Emerged adult mortality; DEA= number of emerged adult dead ; EA = number of emerged adult;
- EAMyc (%) = CEA*100/DEA; where EAMyc = Emerged adult mycosis; CEA= number of emerged adult colonize by fungus ; DEA = number of emerged adult dead;
- CMort (%)= (DP + DEA)*100/ITP; where Cmyc= combined mortality; DP= number of dead pupae; DEA= number of emerged adult dead; ITP = number of initial treated pupae;
- CMyc (%)= (CP + CEA)*100/ITP; where Cmyc= combined mycosis; CP = number of surface colonized pupae; DEA= number of emerged adult dead ; ITP = number of initial treated pupae.

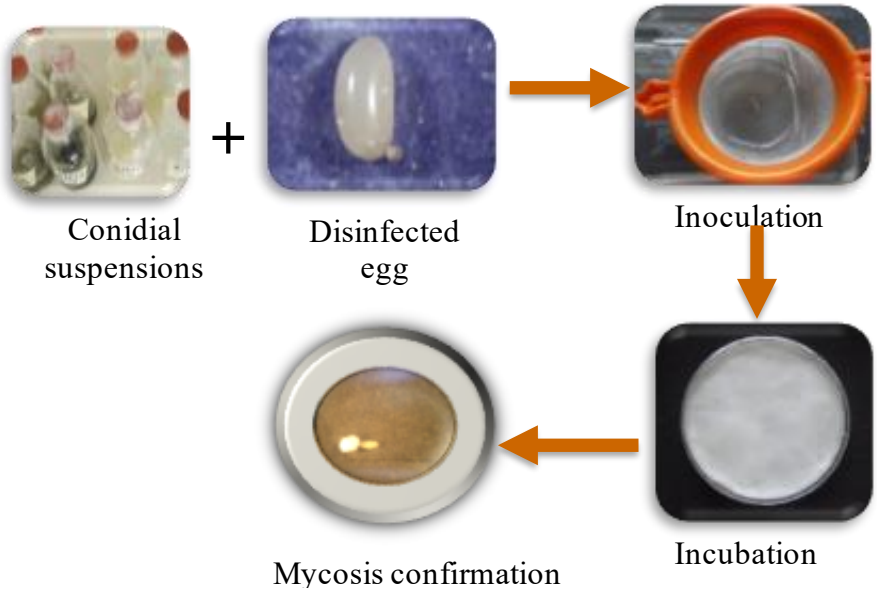

Fig. 12. Steps for egg treatment with fungal suspensions.

II.3.1.2. Endophytic colonization test

The banana planting materials (one-year-old) produced following the method of Vuylsteke (1998) and planted singly in small plastic bags containing sterilized black soil and sand (ratio 3:1) were provided by banana tissue culture nursery management of IITA-Cameroon. The conidia suspensions of the six fungal isolates mentioned above were tired at 3.2 x 10^8 conidia/ml and apply to the banana plantlets. The applications of suspensions were done using 3 different methods: (1) injection into the plant, (2) foliar spray, and (3) immersion of banana corm pieces (Appendix 2).

II.2.1.2.1 Injection of conidial suspension into the plant

In this method, banana tissue culture plants rhizome were injected with 2 ml suspensions using sterilized insulin injection needles (Prabhavathi, 2012). Plants injected with 2 ml of a sterile solution of tween 80 (0.1 %) were considered as control. Treatments were replicated three times. After inoculation, plants were kept in the greenhouse for four weeks (Akello et al., 2008) and watered as needed (Parsa *et al.*, 2013). Each plant was uprooted four weeks post-inoculation and washed with tap water and examined if the entomopathogenic fungal isolates were endophytes. Leaves of each plant were sterilized under a laminar airflow

cabinet by dipping in 5% sodium hypochlorite and 75 % ethanol for 1 min, then cut into small pieces of equal-sized (Akello *et al.*, 2007; Prabhavathi, 2012). The pieces were rinsed thrice in sterile distilled water, then cut into smaller pieces (0.4 – 0.5 cm long) with a sterilized scalpel blade. Five pieces cut from each sampled leave were placed in 99 mm PDA plates supplemented with 0.2 g streptomycin sulfate added as an antibiotic (Wilson and Stanley, 2001). Each treatment had two plates of five leaves pieces per plant. Plates were incubated in the dark at 25 °C for 20 days with a visual examination of fungal outgrowth every 2 - 3 days (Parsa *et al.*, 2013). A fungal colony was characterized as *B. bassiana* based on white dense mycelia, becoming cream to pale yellow at the edge (Humber, 2012). In all cases where there was fungal growth with potential confusion with other fungal taxa, both mycelium and conidia were observed under a microscope using a mounted slide containing fungi structures and water drop. *Beauveria bassiana* features were characterized based on the identification key described by Humber (2012). Percentage colonization was calculated as follow:

C= PPF*100/TPP; where C= percentage of colonization; PPF= number of plant pieces exhibiting fungal growth; TPP= total number of plant pieces.

II.3.1.2.2. Foliar spray of conidia suspension

For the foliar spraying method, one banana tissue culture plantlet of one year with at least four leaves were used for fungi application or 0.1 % Tween 80 (control). The application consisted of spraying the conidial suspension of each fungal isolate or Tween 80 solution on the axial (upper) surface of leaves using a manual atomizer, until saturation. During application, the top of the pot was covered with aluminum foil to avoid conidial runoff to the soil (Parsa *et al.*, 2013). Treatments were replicated three times and plants were transferred in greenhouse for four weeks before examination of fungi colonization on leaves as described for the injection application method (Akello *et al.*, 2008).

II.3.1.2.3. Banana corm inoculation with a conidial suspension

Banana plant corm of local variety Asung-begle (variety choice based on availability) was trimmed, cut into pieces, disinfected with 1 % hypochlorite, and rinsed thrice in sterile distilled water. The disinfected corm pieces were immersed individually in the conidial suspension of each isolate with 3 repetitions, placed in Petri dishes then sealed with parafilm.

The dishes were then transferred in the incubation chamber in the dark at 25 °C and relative humidity 70 – 80 %. Cuttings treated with tween solution (0.1 %) were considered as control. Two weeks post-incubation, corms were disinfected and trimmed using a sterile blade and separated into three batches. For the first batch, slices of corm tissue were observed under a microscope slide at magnification X 40; the second batch was directly cultured on PDA media using a method described for the examination of fungi colonization on leave. Fungal colonies were characterized based on the Humber key of identification (Humber, 2012). The percentage of colonization was calculated using the formula described above C). The third batch of plant pieces was pounded in a mortar, and then 5 ml of the extract were added on the pounded disinfected corm to be given as food to larvae. The larvae were inspected every two days for fourteen days and dead larvae were incubated on wet filter paper to favor fungal outgrowth and visual identification. The percentage of larvae killed by the fungus was calculated with the following formula: LMyc (%) = CL*100/DL; where Lmyc = Larval mycosis; CL = number of surface colonized larvae; DL = number of dead larvae.

II.3.2. Epizootic potential of thermal tolerance of fungal isolates

II.3.2.1. Effect of temperatures on fungal isolates

The thermal response of fungal isolates was determined by assessing the effect of seven constant temperature on biological parameters such as germination, mycelial growth, and conidia production.

II.3.2.1.1. Germination test under constant temperatures

Conidia of each isolate were harvested from the surface of a 15-day-old pure culture of each isolate and suspended in 0.1 % Tween 80. The suspension was then stirred with a vortex mixer and filtered with a sterile cheesecloth before conidia counting under a dissecting microscope using a Malassez cell (Marienfeld, Germany). Conidia viability of each isolate was determined by spraying 0.1 ml of conidia suspension label 3.2×10^6 conidia/ml on PDA plates using an inoculation loop. Four replicates were established for each isolate. Covered glass Petri dishes were tightly sealed with parafilm and transferred to climate control chambers (Percival Scientific, USA) set at 7 constant temperatures (13, 15, 20, 25, 29, 33, and 37 °C), 70-80 % relative humidity, and total darkness. The 7 constant temperatures were

chosen to cover the range of temperatures in regions with banana and plantain cultivations worldwide (above 13 °C and under 38 °C) and where BRB occurs (above 12 °C and under 34 °C) (Traore *et al.*, 1993; Newley *et al.*, 2008; Duyck *et al.*, 2012). The number of germinated conidia was counted on a total of 400 spores (100 spores from each of the four sections) at 40X magnification (Leica, Germany). Conidia were considered as germinated when the germinal tube became longer than normal conidia (Petlamul and Prasertsan, 2012). Germination percentage was calculated using the formula: G = GC*100/ TC; where G= percentage of germinated conidia; GC= number of germinated conidia; TC= total counted conidia.

II.3.2.1.2 Mycelial growth and conidiation under constant temperatures

To determine the radial growth capacity of each isolate under the 7 constant temperatures, a 4-mm colony disc of each isolate was removed from the same growing plate of 3 to 5-day-old pure culture incubated at 25 °C and placed in the center of Petri dishes, similar to the description of this process in Petlamul and Prasertsan (2012). Inoculated plates were sealed with parafilm and transferred for incubation at the same conditions set for conidia germination. There were 4-5 replicates per isolate at each of the 7 temperatures. The radial growth (mm) in each plate was measured daily for 21 consecutive days by using two perpendicular diameters (mm) drawn on the bottom of the Petri plates (fig. 13). The formula of Mamza *et al.* (2008) was used to calculate radial growth: D = (D1 + D2/2)-D0; Where D_1 = diameter of line 1 (mm), D_2 = diameter of second line (mm) and D_0 = diameter of the explant (mm).

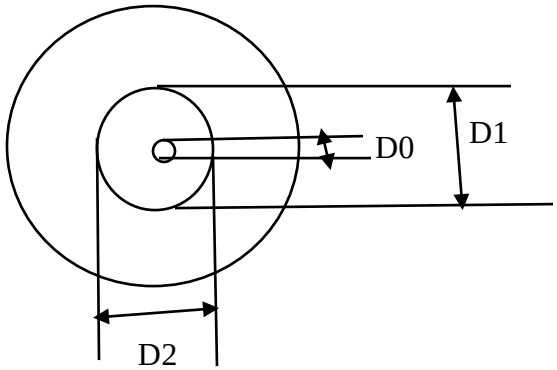

Fig. 13. Principle of radial growth measuring.

Fungi colony from radial growth evaluation at day 21 post-inoculation was used for conidiation determination. Conidia each plate from were harvested by scraping the media surface with a sterile scalpel and suspending the whole colony in 10 ml of 0.1 % Tween 80 solution. Conidia yield was estimated by diluting the conidia suspension and counting the number of spores with a hemocytometer under a light microscope at 40X magnification (Petlamul and Prasertsan, 2012).

II.3.2.2. Horizontal transmission test and conidiation on insect

Horizontal transmission potential was determined for *B. bassiana* isolates BIITAC6.2.2 and BIITAC10.3.3 and for *M. anisopliae* isolates MIITAC6.2.2 and MIITAC11.3.4, which had the broadest temperature range for germination and sporulation (see results section). The horizontal transmission was evaluated using fungus-treated and control (uninfected) adult weevils in four-vector ratios (i.e, percentages of fungus-treated weevils) of 0, 10, 30, and 50 %, with a total of 30 insects in each of 4 replications per isolate (Lopes *et al.,* 2011). Fungus-treated weevils were air-dried under room temperature after marking them with white paint (Nr. Igle-147) on their dorsum, then dipping them individually for 30 seconds in 0.1 ml conidial suspension of each isolate prepared at a concentration of 3.2 x 10^8 conidia/ml. Control insects were dipped in a solution of Tween 80 for 30 seconds. Both fungus-treated and control insects were placed at the diagonally opposite corners of a sterile plastic container (7 x 5 cm) containing 20-g pieces of plantain corm as food which was changed at 5-day intervals. Containers were incubated in laboratory conditions: darkness, 70-80 % relative humidity, and 25 °C, which is the optimal temperature for adult weevil activity (Cuillé, 1950. Dead, non-inoculated insects were removed and incubated on moist filter paper in Petri dishes at the condition mentioned above to favor mycosis development.

To evaluate conidiation on insects, ten cadavers of non-inoculated insects for each isolate, presenting signs of infection by the pathogens after seven days (i.e., insect from mycosis confirmation test) were randomly collected to determine conidial yield per cadaver. This process consisted of transferring cadavers individually in a 10 ml sterile solution of 0.1 % Tween 80 and shake for 3 min using vortex to break down the chain and facilitate dispersion of conidia (Latifian and Rad, 2015). The suspension was then serially diluted, and spores were counted under Haemocytometer Malassez cell to estimate conidial yields.

II.3.3. Evaluation of the effect of some environmental factors on the development of *B. bassiana* and *M. anisopliae in vitro*

The response of two isolates, one *B. bassiana* (BIITAC6.2.2) and one *M. anisopliae* (MIITAC11.3.4) to pH, photoperiod, UVB light, and relative humidity was assessed by evaluating biological parameters such as conidia germination, mycelial growth, and conidiation.

II.3.3.1 Effect of pH levels on entomopathogenic fungal isolates

The PDA media was prepared, and pH ranked 2 - 14 was adjusted using 1M HC1 or 5M NaOH. The medium prepared in different pH was sterilized at autoclave 125 °C for 20 min and antibiotic, streptomycin was added when the medium cooled at 55 ◦C. The media were used to determine conidia germination; their mycelia growth and sporulation of strain BIITAC6.2.2 and MIITAC11.3.4.

For the germination test, conidial suspensions prepared from a 14 days' pure culture of each isolate were quantified using a hemocytometer and diluted with a solution of tween 80 (0.1 %) to obtain conidial suspension tired 3.2 x 10^4 conidia/ml. Forty microliters of conidial suspension of each isolate were spread onto PDA (8 ml) plates of different pH with five replicates. The plates were transferred in a culture chamber at continuous dark, 25 ℃ and 70 – 80 % RH. After 4 and 10 days of incubation, the number of colonies was counted and registered.

Mycelia growth of the fungal isolates was evaluated by placing 4 mm disc of a two to three days' pure culture at the center of a PDA plate of different pH with four replicates. Cultures were incubated at 25 °C, in dark and 70 – 80 % RH, and the colony was measured from the second day by cross-measuring of the diameters (Mamza *et al.*, 2008). Colony diameters were taken daily for 15 days (Padmavathi et *al.*, 2003; Otgonjargal et *al.*, 2015). Mycelia growth rate (Hallsworth and Magan, 1996) was calculated as followed:

M= D/T; where M= mycelia growth; D = radial growth; T= incubation day

After 15 days, conidia were scrapped on each plate and suspended in 10 mL aqueous solution of tween 80 (0.1 %). Then the concentration of conidia in each suspension was determined using a hemocytometer (Weijia Technology Company, China).

II.3.3.2. Effect of UVB light on entomopathogenic fungal isolates

Forty microliters of each fungus isolate inoculum of 3.2 x 10^4 conidia/ml (prepared as described above) were sprayed on PDA media prepared at normal pH (5.6 ± 0.2). The plates containing the treatments were introduced in the trans-illuminator (fig.14) at exposure times 0, 15, 30, 45, and 60 min. Then the plates were transferred in a culture chamber at photoperiod 0:24 light/dark, 25 °C and 70 – 80 % RH for 4 and 10 days. At the end of incubation time, the number of colonies was counted and recorded. Control conidia were not irradiated.

The mycelial growth and sporulation were also evaluated as described above (II.3.3.3). Four 4 mm disc of a two to three day obtained from a pure culture of each strain was placed on the center of the plate containing culture media (normal pH of 5.6 ± 0.2). The plates were sealed with parafilm and transferred in the transilluminator for different exposure times (15, 30, and 45 min). After the exposure time, cultures were incubated at 25 °C, in dark and 70 - 80 % RH and the two diameters of fungal colonies drawn on each plate were assessed daily from the second day till the 15th day. Four replicate dishes per exposure time were irradiated in each trial but the control was not irradiated. Mycelial growth rate was calculated as described above.

Conidia production of each isolate was also determinate by counting the number of conidia in conidia suspension prepared by scrapping conidia on each plate surface and suspended in 10 ml aqueous solution of tween 80 (0.1 %) (Mweke *et al.*, 2018).

Fig. 14. Fungal isolates under UV trans-illuminator.

II.3.3.3. Effect of photoperiod on entomopathogenic fungal isolates

Plates containing PDA media (at normal pH of 5.6 ± 0.2) were divided into two batches. The first batch was inoculated with the forty microliters conidial suspension of each isolate prepared at 3.2 x 10^4 conidia/ml; and in the second batch plates, a 4 mm disc from 2 - 3 days' pure culture was placed on the center of a dish. All the plates were transferred in different photoperiod condition: continuous light (24:0 L: D), 12 h light and 12 h dark (12:12 L: D), and continuous dark (0:24 L: D) (Pittarate *et al.*, 2016), 25 °C and 70 – 80 % RH. Each photoperiod had four repetitions.

The first batch plates were inspected after four days and the number of colonies per plate was counted. For the second batch plates, colony diameter was measured daily from day 2 to day 15. The conidia yield was determined from plates used for mycelia growth evaluation as described in the previous section. Each photoperiod was repeated four times per isolate in both batches.

II.3.3.4. Effect of relative humidity on entomopathogenic fungal isolates

PDA plates (at normal pH of 5.6 ± 0.2) were divided into two batches. The first batch was used to assess conidia viability and the second batch, mycelia growth and conidia production.

For assessment of conidia viability, plates were inoculated with forty microliters conidia suspension of each isolate prepared at 3.2 x 10^4 conidia/ml and incubated in dark at 25 °C and different relative humidities 35, 50, 75, and 99 %. Each isolate had four replicates per relative humidity. The number of colonies was counted on each plate after four days.

A disc of 4 mm colony from two to three day's pure culture of each isolate was placed at the center of the second batch plates. The plates were transferred in the continuous dark at 25 °C and different relative humidity conditions (35, 50, 75, and 99 %). From the second day post-incubation, the colony diameter was measured daily for 15 days. Each isolate was replicated four times relative humidity.

Conidia production was assessed using conidial suspension obtained by dipping conidia from 15 days' culture (used for mycelia growth evaluation) in 10 ml Tween 80 solution. The assessment consisted of counting of the conidia number in 1 ml suspension of each isolate. Four replicates were performed for each relative humidity per isolate.

II.3.4. Selection of ingredients for biopesticide development

II.3.4.1. Quality control test of mass-produced conidia

For quality control test, conidia of the two candidate entomopathogenic fungi *B. bassiana* and *M. anisopliae* respectively BIITAC6.2.2 and MIITAC11.3.4 were re-isolated on adult banana weevil *C. sordidus* and cultured on PDA medium. Harvested conidia on the PDA plate surface were suspended on sterile distilled water and sent to IITA-Cotonou (Benin) for conidia mass production using long grain rice fermentation technic.

One hundred and fifty grams (150 g) of rice was autoclaved with 60 ml of sterile water in autoclavable polyethylene bags (10x33 cm) for 15 min at 121 °C and pressure of 1.05Kg/cm^3 (Nufroho and Ibrahim, 2007). During the autoclaving period, bags were loosely sealed with 43cm polyvinyl chloride pipe (3 cm diameter) and plowed with cotton. One milliliter (1ml) of 1x10^8 conidia ml suspension in 0.1 % aqueous Tween 80 was introduced to each bag containing cool sterilized rice (24h). The bags were left in the dark at 26 ± 1 °C for 14 days. The 14 days old fermented culture was harvested and the colonized substrate was spread evenly to air-dry and sandwich between paper towels for 4 days to induce full sporulation. The dried conidia were sieved with a 125µm particle size sieveBelloa et *al.* (2000). One gram of dried conidia was quantified by dipping 1g of air-dried conidia in 10 ml aqueous solution of tween 80 (0.1 %), using a hemocytometer. The number of conidia was then determined followed by conidia viability through the germination test as described above.

II.3.4.2. Screening of carriers for powder formulations

Organic ingredients: Carrot flour, banana flour, maize flour, rice flour (Latifian *et al.*, 2013), gari powder, Baobab fruit powder (*Adansonia digitata*), and inorganic materials as Charcoal, wood ashes, and kaolin clay were used as conidia carriers. Other ingredients like milk powder and Mucuna dry leaves powder were selected for their richness in carbohydrates and nitrogen. Pure air-dried conidia of each entomopathogenic fungi from Cotonou were mixed with the sterilized carrier at a ratio of 1:9 (10 % w/w a.i.) under laminar flow. Then tests were performed with the preliminary formulations to determine compatibility between conidia and carriers (shelf-life) and characterize carriers, for suitable management of banana and plantain bio-aggressors.

II.3.4.2.1. Assessment of the compatibility of carriers with conidia

Each preliminary formulation obtained by mixing BIITAC6.2.2 or MIITAC11.3.4 with the 12 sterilized carriers was stored at 4 °C and the germination ability of stored conidia from different formulations was assessed one day after formulation to obtain the initial germination level and at intervals of 4, 8, and 12 weeks. Unformulated conidia stored in the same conditions were considered as control. To evaluate the persistence of spore viability over time, 0.1 ml aliquots containing 3.2 x 10^6 conidia/ml from each stored condition was spread on PDA plates, and plates were incubated in dark for 16 hours before assessment of germination rate on 400 conidia as described above (Moore *et al.*, 1993).

II.3.4.2.2 Water holding capacity or porosity

The water holding capacity of samples was determined according to the methods of Shad *et al* (2013) in a centrifuge at room temperature with slight modification.

Distilled water (30 ml) was added to 3 g of each sample contained in a 50 ml centrifuge tube (ingredient) and mixed with a blender (vortexed genie 2™) for 30 s. After allowing samples to stand for a further 30 min for hydration, centrifuge tubes well covered were transferred into centrifuges bowl at 2000 rpm for 10 min. Then after draining the supernatant, each centrifuge tube containing only the sediment was weighed and WHC (%) was calculated as follow (El Demery, 2011):

WHC = (WHS – WDS)*100/WDS where WHC= percentage of water holding capacity; WHC = weight of hydrated sample; WDC = weight of the dry sample.

II.3.4.2.3 Physical uniformity

In this assay, flow properties and granulometry were determined. For flow properties, the untapped volume and tapped volume of 5 g of each sample were measured in a measuring cylinder (50 ml) respectively before and after tapping the cylinder on a wooden plank until no visible decrease in volume was noticed (Chandra and Samsher, 2013**)**. Hausner ratio and Carr's index were determined based on the weight and volume as described by Jones *et al.* (2000) with the following formula:

TD = WS/ TV where TD = tapped density; WS = weight of sample; TV = tapped volume

BD = WS/ UTV where BD = bulk density; WS = weight of sample; UTV = untapped volume

HR = TD/ BD where TD = tapped density; BD = bulk density;

CI = (TD – BD)/ TD where CI = carr's index; TD = tapped density; BD = bulk density.

For granulometry, particle size distribution was determined using Cavalcanti *et al* (2012) sieving method with some modifications. This consisted of sieving 5 g of the carrier through sieves of 2000, 500, 150, 100, 38, and 28 µm for at least 5 min. After sieving, the quantity of material retained was weight and the percentage of cross material was calculated: CM = (IW – RW)*100/ IW where CM = percentage of cross material; IW = initial weight; RW = retained weight**.**

II.3.4.2.4 Neutral pH

The pH of ingredients was measured using a pH-meter (accumet ^{R} AB^{15} PH meter, Fisher scientific) (Tran Van Cuong and Koo Bok Chin, 2016). It was determinate by adding 4 g of sample to 40 ml distilled water and stir for 10 min (fig. 15B). A buffer 4 - 7 was used to standardize. Three replicates were made for each ingredient.

II.3.4.2.5 Moisture content

The moisture content of each carrier was measured three times using an humidimeter (fig. 15 C).

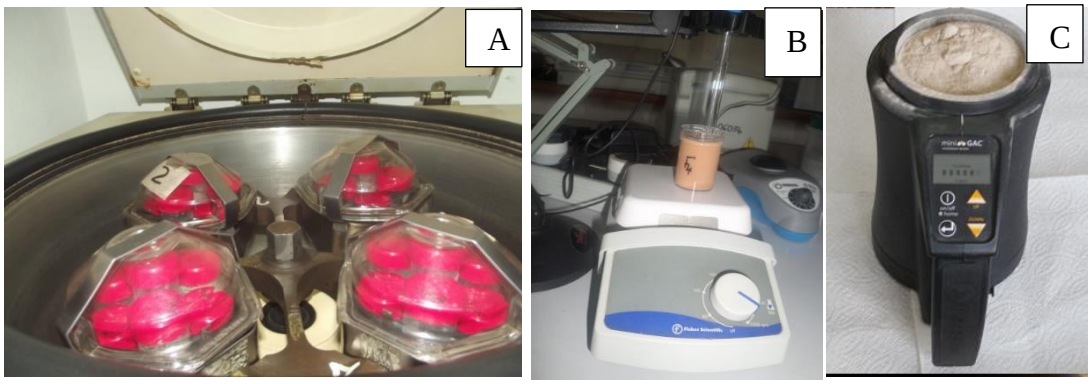

Fig. 15. Physicochemical assessment of pre-formulation under A) centrifuge, B) pH meter, and C) hygrometer.

II.3.4.2.6 UV action

Potential photo-protectant was selected after exposure of one gram of pre-formulations (made of each ingredient) for 1 hour under UVB light in UV trans-illuminator at direct natural sunlight or UV trans-illuminator at mid intensity. Ingredients showing high tolerance to UV light were also exposed to direct natural sunlight at mid intensity for 1 hour **(**Edgington *et al.*, 2001).

Following exposure, each pre-formulation was suspended in 10 ml sterile distilled water and 0.1 ml suspension of each formulation at concentration 3.2 x 10^6 conidia/ml was inoculated on Petri dishes containing PDA medium with five repetitions. Then plates were placed in the dark for 16 hours at room temperature. Conidia germination was determined using a microscope. The mean percent spore germination for 400 conidia was calculated for each slide by counting the number of germinated and non-germinated conidia in four fields of view as above.

To quantify the efficiency of each ingredient tested, the degree of spore inactivation was quantified using the formula of the percentage of original activity remaining (OAR), a standard parameter used for quantifying UV inactivation of entomopathogens (Shapiro *et al.*, 1983; Martigoni and Iwai, 1985; Ignoffo and Garcia, 1994; Patel *et al.*, 1996):

OAR= GEC*100/ GUC; where OAR= original activity remaining; GEC= percentage gemination of exposed conidia; GUC = percentage germination of unexposed conidia.

II.3.4.2.7 Availability and economical value

Three different markets around Nkolbisson were chosen at random to check whether the ingredients used for preliminary formulations were present or not and what was the price of a kilogram. The ingredients bought less than 1kg were converted to have the price of one kilogram.

II.3.5. Characterization of biopesticide formulations

II.3.5.1. Some physicochemical characteristic of formulations

Ingredients such as desiccant (6.67 %), salts (0.02 %), UV protectant (3.99 %), stabilizer (1.99 %) and carrier (74 %) (Nugroho and Ibrahim, 2007; Mbarga *et al.*, 2014) were homogenized using a Convivium Mixer for 10 min. Four co-formulations were obtained using

kaolinite clay (Co-F1), maize flour (Co-F2), banana flower (Co-F3), and cassava starch (Co-F4) as carriers. The composition of each powder-based biopesticides is resumed in table I below.

Table I. Composition of co-formulations

Composition	Co-F1	Co-F2	Co-F3	Co-F4
Carrier (74 %)	Kaolin clay	maize flour	banana flour	cassava starch
Desiccant (6.67 %)	silica gel	charcoal	silica gel	silica gel
Salts (0.02 %)	Cacl2	Cacl2	Cacl2	Cacl2
UV protectant (3.99 %)	banana flour	kaolin clay	kaolin clay	kaolin clay
Stabilizer (1.99 %)	wood ashes	wood ashes	wood ashes	wood ashes

Co-F1= co-formulation based on kaolinite clay; Co-F2= co-formulation based on maize flower; Co-F3= co-formulation based on banana flower; Co-F4= co-formulation based on cassava starch.

The water holding capacity of each co-formulation was determined according to the methods of Shad *et al* (2013) described above. Flow properties (Hausner ratio and Carr's index) were also estimated using the method of Jones *et al* (2000). pH meter and hygrometer were used to measure the pH and moisture content of each co-formulant as described above.

II.3.5.2 Shelf-life of conidia at different storage conditions

Biopesticides formulations were prepared by adding 13.33 % technical powder (*B. bassiana* isolate BIITAC6.2.2) in each co-formulation (Nugroho and Ibrahim, 2007; Mbarga *et al.*, 2014). Four formulations (20 g each) were obtained using as carriers, kaolinite clay (F1), maize flour (F2), banana flower (F3), and cassava starch (F4) as described in Table II. One gram of each formulation was collected for direct assessment of conidia viability while the 19 g of each formulation and unformulated conidia was poured into 50 ml falcon plastic tubes and covered with aluminum foil before storing under different temperatures conditions of -50 °C, -20 °C, 4 °C and 25 °C. The conidia viability of formulated and unformulated conidia was assessed every four weeks for 72 weeks (Mbarga *et al.*, 2014 modified). Every four weeks, 1 ml formulation suspension at concentration 3.2 x 10^6 conidia was spread in five PDA plates and transferred in incubation chamber at 25°C, 70 - 80% relative humidity in the

total darkness for 16 h. After 16 h of incubation at ambient temperature, the number of germinated conidia on 100 conidia per section in a plate was recorded. Each plate was separated into four sections. Conidium with its germ tube longer than normal conidia was considered as germinated. Germination percentage was calculated using the formula : G = GC*100/ TC ; where G = percentage of germinated conidia ; GC = number of germinated conidia ; TC = total counted conidia.

Table II. Composition of biopesticide formulations

Composition	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Technical powder (13.33 %)	BIITAC6.2.2	BIITAC6.2.2	BIITAC6.2.2	BIITAC6.2.2
Carrier (74 %)	Kaolin clay	maize flour	banana flour	cassava starch
Desiccant (6.67 %)	silica gel	charcoal	silica gel	silica gel
Salts (0.02 %)	Cacl2	Cacl2	Cacl2	Cacl2
UV protectant (3.99 %)	banana flour	kaolin clay	kaolin clay	kaolin clay
Stabilizer (1.99 %)	wood ashes	wood ashes	wood ashes	wood ashes

II.3.5.3 Effect of adjuvants and formulations against banana weevil in the laboratory

The effect of carriers, as well as the effectiveness of formulations, was assessed through direct contact with banana weevil adults. The co-formulants or adjuvants selected for screen-house assay were mixed with (T5, T6, T7, and T8) and without conidia (T1, T2, T3, and T4) then 5 adult weevils were released in each treatment with six replicates according to the method of "Walk on colony process'' described by Nankinga (1994) and used by Aby *et al* (2010). It consisted of making living adults weevil obtained from the laboratory rearing, walk in the powder (formulation or mixture of co-formulant) contained on the Petri dish (Appendix 3). Dead weevils were recorded daily and incubated on moist filter paper for mycosis confirmation. Food made of a piece of banana corm was changed once a week. Treatments were:

T_0 = no conidia; T_1 = formualtion1 without conidia; T_2 = formulation 2 without conidia; T_3 = formulation 3 without conidia; T_4 = formulation 4 without conidia; T_{0+} = = only conidia; T_5 =

formulation 1 with 10^{10} conidia/ml; T_6 = formualtion2 with 10^{10} conidia/ml; T_7 = formulation 3 with 10^{10} conidia /ml; T_8 = formulation 4 with 10^{10} conidia /ml.

II.3.5.4. Effect of formulations in the greenhouse conditions

II.3.5.4.1. Experimental design

For the pot experiment conducted at IITA station (October 2015 to March 2016), bioassay (fig. 16A and 16B) was established for evaluation of biopesticide formulations efficacy when treated before pre-infestation method) and after infestations (post-infestation method). Sword suckers of the local variety "Asung-begle" obtained from farmers were used for the experiment. Sterilized suckers (hot water treatment) were planted in 15 l buckets containing sterile soil (Gold *et al.,* 1998). The buckets were arranged in a completely randomized design with ten plants per treatment and grouped into two subsets of five buckets per treatment (Godonou *et al.*, 2000; Tumuhaise *et al.*, 2003, Kosma *et al.*, 2012). Treatments consisted of (fig.16 A and 16 B):

- untreated and un-infested plantain suckers (T0-);
- untreated and infested plantain suckers (T0+);
- T1, T2, T3, and T4 where formulation based on kaolinite clay, maize flour, banana flour, and cassava starch respectively were applied as the post-infestation method (Applic 1);
- T1', T2', T3' and T4' where formulation based on kaolinite clay, maize flour, banana flour, and cassava starch respectively were applied in the pre-infestation method (Applic 2).

For the pre-infestation method (fig.17C), 10 g of each formulation was poured per planting hole, where un-infested plantain sucker was planted. Each formulation had ten repetitions, a plant considered as a replicate. Fourteen weeks after planting (14 WAP) 5 males and 10 females were released at the sucker's mat into each pot (Godonou *et al.*, 2000).

For application using the post-infestation method (fig.17B), plantain suckers were planted without formulation. Fourteen weeks after planting (14 WAP) adult weevil (5 males and 10 females) were released on the sucker into each pot where each formulation was added two weeks later using the methodology of Magara *et al* (2003) with modification. To prevent

the escape of the weevil, each pot was covered with mosquito nets (fig. 17 A) and watered twice a week as necessary.

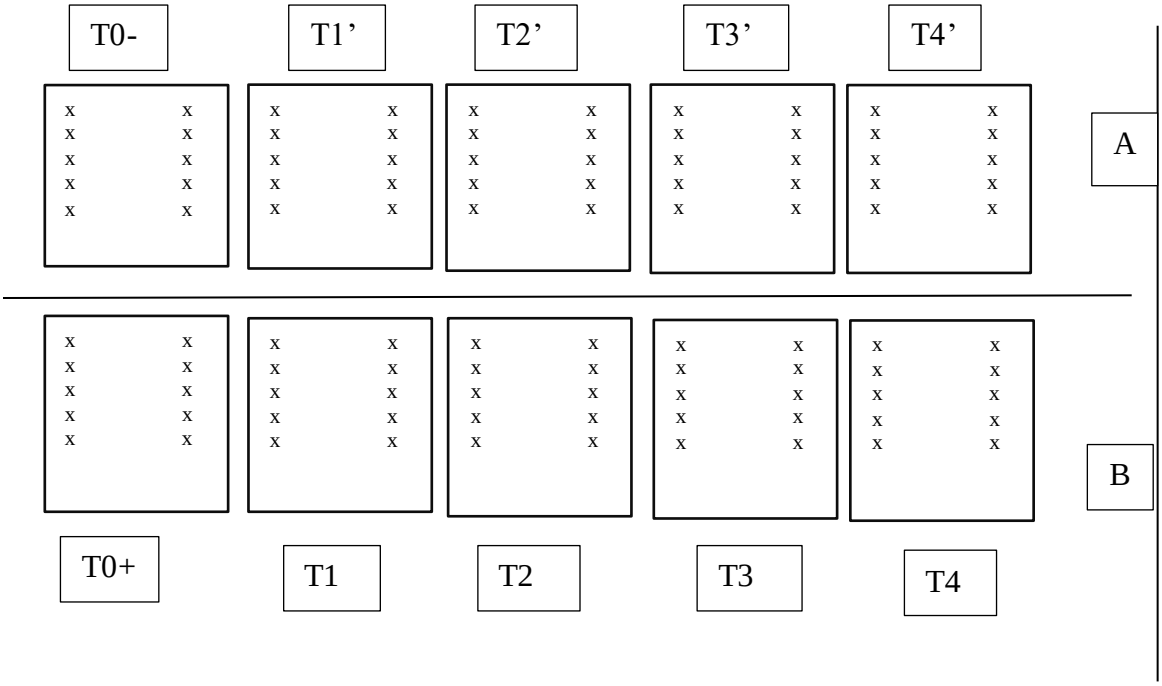

Fig. 16. Experimental design in the screen-house: controls (T0- and T0+); formulation1 (T1 and T1'); formulation2 (T2 and T2'); formulation3 (T3 and T3'); formulation4 (T4 and T4'); A) application as pre-infestation method; B) application as a post-infestation method.

II.3.5.4.2. Data collection

Data such as the temperature of the greenhouse, the moisture content in pots, growth parameters, insecticidal potential parameters, conidia persistence, and damage on banana corm and reproduction of *C. sordidus* were collected.

Greenhouse temperature and moisture content in pots from planting day still the end of the experiment were determined using a thermometer and moisture meter at 2.5 cm depth (Magara *et al.*, 2003).

Allometric data such as circumference of the pseudo-stem at 2 cm height, height of the plant (distance from the base of the plant to the youngest leaf axil), number of fully developed and functional leaves (defined as > 50 % green), width of the leaf (the widest area from the lamina) and length of the leaf (distance from the leaf apex to the leaf stalk) of the youngest

fully opened leaf was recorded on each plant every two weeks during the trial from 8 WAP to 22 WAP (Smithson *et al.*, 2004; Akello *et al.*, 2008).

From seventeen weeks after planting (17 WAP) to 22 WAP, cadavers of weevil were monitored in each post on a weekly basis and incubated on filter paper for mycosis confirmation after disinfection with 1 % hypochlorite (Akello *et al.*, 2007). At the end of the assay, the soil in each bucket was inspected for all living and dead adult banana weevil. Then, the mortality rate of adult weevil, as well as infection rate, were calculated using the formula (Akello *et al.*, 2008):

- AMort (%) = DA*100/ITA; where AMort = adult mortality; DA= number of dead adult; ITA = number of initial treated adult;
- AMyc (%) = MA*100/TD; where AMyc = adult mycosis; MA= the number adult weevil showing fungi outgrowth; TD = total mortality per plant

Conidia persistence was also assessed by collecting soil samples at 2 months, 4 months, and 6 months post-inoculation and releasing three to five banana weevil larvae in each soil sample. Mortality was assessed every 2 days for 14 days. Dead weevils were incubated in wet filter paper for mycosis confirmation. When an insect showed signs of infection, conidia were considered viable and persistent.

For evaluation of the damage on banana corm and reproduction of *C. sordidus*, plants were removed from the soil at the end of the experimental period and washed with tap water, then pseudo-stem cut at a height of 30 cm. The bulb (corm) was carefully trimmed with a knife and eggs removed with a sharp-edged knife. These eggs were counted. The damage scoring methods described by Gold et *al.* (1994) was used for assessment of banana weevil damage on the peripheral and internal part of all the plant after harvesting. This method consisted of trimming and dividing the rhizome into 4 equal parts (25 %) then bringing it to the surface or area (m^2) of the rhizome dug by the larva outside the surface for each of the four portions. Thus, the percentage of damage is estimated from the 4 portions. To evaluate the damage of the base of the pseudo-stem and the rhizome, cross-sections were made on the "collar" (meeting point between rhizomes i.e. 2 cm below the "collar" respectively). For each cross-section, the central cylinder and cortex were divided into 4 equal parts each respecting 25 % of the area (m^2). The percentage of the area of each portion consumed by the larvae of weevil was estimated for each 25 % piece and added to obtain the percentage of internal

damages, the external damages of the central cylinder and cortex respectively. The number of weevils at the immature stage (larvae and pupae) found in corm where damages were assessed, was registered per plant and treatment i.e. a number of dead and living larvae and pupae. The dead larva or pupae were incubated on humid filter paper in a Petri dish for 15 days to verify for mycosis confirmation. Thus, the percentage of living or dead or mycosis larva and pupa was calculated as described above.

Fig. 17. Greenhouse trial: A) handling of buckets; B) post-infestation method; C) pre-infestation method.

II.3.5.5. Effect of formulations applied alone in the field conditions

II.3.5.5.1. Site description and experimental design

An experimental setup was placed at IITA station-Nkolbisson (14m x 33m or 462m^2) with a randomized complete block design with four replicates (fig.18). Banana mats were spaced in a 3 x 3m arrangement (appendix 5). Planting was made by adding animal manure as describe by Gold *et al* (1998). Then, pseudo-stems traps were placed to evaluate the population from natural infestation before treating the site.

Treatments were: (1) control; (2) formulation1; (3) formulation2 (fig. 18); thirty grams of each formulation were applied in 0.75 mm deep by hand on the soil surface around the banana mat at the distance of 0 m radius (Nankinga and Moore, 2000; Schoeman and Botha, 2003). The banana trash mulch was removed before the application of the formulations then replaced to cover the applied formulations (Nankinga and Moore, 2000). Spraying was done in the late evening to minimize the possible suppressive effect of the sun's rays on the conidial germination.

For soil fertility, animal manure was applied four times a year (Smithson et *al.*, 2004). De-suckering was made subsequently every one or two months by cutting excess sucker at their bases or removing them, to maintained plant density at three plants per mat i.e.one flowered, one pre-flowered, and one sucker (Rukazambuga *et al.*, 2002; Masanza , 2003).

Plantations with Cameroon local varieties Asung-begle were favored during the selection of the experiment site.

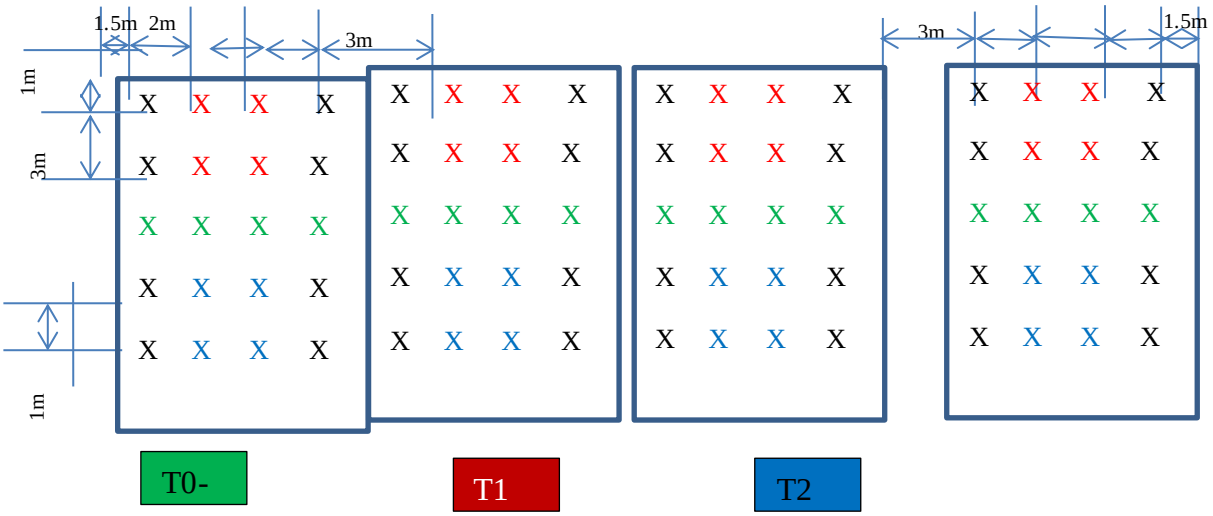

Fig. 18. Experimental design at IITA station-Nkolbisson with T0=control, T1= formulation1 and T2 = formulation2.

II.3.5.5.2 Data collection

Data such as growth parameters, weevil damage, insecticidal potential, conidia persistence, mycosis of non-target, and environmental factors were collected.

II.3.5.5.2.1 Growth parameters and damage evaluation

Plant growth described above, and yield were collected during the first crop cycle. The daily inspection was made to detect flowering, then plant girth (measure 50 cm above ground level), height (distance between the soil surface and the base of the upperm ost leaf), and the number of functional leaves was recorded for all plants within one week of flowering.

When plants reached physiological maturity i.e. ripening of the first finger, it was harvested, and bunches weight measured using a balance (Rukazambuga *et al.*, 2002; Gold *et al.*, 2005; Musanza *et al.*, 2006). A score of 0 kg was given to the plant dead without producing

a bunch. Yield loss damage was estimated based on bunch size and damage of the central cylinder. Plants were grouped as follow to test the effect of *C. sordidus*: (1) low damage (0 – 5 %); (2) moderate damage (> 5 – 10 %); (3) heavy damage (> 10 – 15 %); and (4) very heavy damage (> 15 %).

II.3.5.5.2.2. Evaluation of the insecticidal potential of formulations against the weevil population

The weevil population was assessed one-week post-application of treatments on the monthly basis, using one pseudo-stem trap per banana mat. The number of weevils was recorded and weevils were released back into banana plants (Nankinga and Moore, 2000; Schoeman and Botha., 2003). At the end of the assay, banana weevil damage on the peripheral and internal parts of all the plants was assessed after harvesting as described above or using the scoring system described by Vilardebo (1973) used by Dassou et al (2015). The scoring system consists of shelling 2 cm of corm surface from 10 cm above to 10 cm below ground over the entire circumference of the corm and scoring damage from 0 - 100 % according to the number of galleries. The scoring was made as follow: 0 = no damage; 5 % = 1 or 2 galleries per corm, and 10, 30, 40, 60 and 100 equaled 10, 25, 50, 75 and 100 % of the corm circumference damaged respectively.

II.3.5.5.2.3. Evaluation of conidia persistence in the field

A Laboratory bioassay was done to determine conidia persistence. Soil samples were collected around the banana plant of control and fungal treatments at 8 months after the second biopesticide application with 3 replicates per treatment. Then, 3-5 disinfected weevil larvae obtained from the rearing unit were exposed to soil samples for 7 days. Mortality was monitored daily for 14 days after exposure and dead weevil were disinfected with 1 % hypochlorite and placed individually on moist sterile filter paper for mycosis confirmation.

II.3.5.5.2.4. Effect on non-target insects

The effect of formulation on non-target insects was monitored by examining and collecting dead insects and insect showing fungal outgrowth under traps, in the ground, and

on trash around plant mat (Hasyim *et al.*, 2009). Dead insects were incubated on moist filter paper for mycosis confirmation.

II.3.5.5.2.5. Evaluation of environmental factors

Environmental factors such as temperature, relative humidity, and rainfall were assessed using hobo and rain gauges (Gold *et al.*, 2004; Duyck *et al.*, 2012).

II.3.5.6. Effect of formulation combined with pheromone baited trap

For the study biopesticide performance combined with pheromone traps, old banana plantation of > 3years old with moderate to the high population of weevil i.e. capture of >1 weevil per trap, and non-use of insecticides were used (Duyck *et al.*, 2012; Fancelli *et al.*, 2013).

II.3.5.6.1. Evaluation of attractiveness of pheromone baited trap

The first part of the study aimed to compare the attractiveness of the pheromone baited trap and pseudo-stem trap. The trial was conducted during the rainy season (long rainy season). Four banana and plantain fields of local and improved varieties were used to conduct the bioassay, two fields for each treatment. Treatments consisted of: i) pheromone baited trap (PS+PH) and ii) pseudo-stem trap (PS) (appendix 6). Three traps were placed per field under a banana mat and inspection was done once a week for four weeks (without changing traps). Pheromone baited trap consisted of hacking one lure on a pseudo-stem trap. A pseudo-stem trap without lure was considered as control (Tinzaara *et al.*, 2005). The distance between traps was at least 36 m.

II.3.5.6.2. Evaluation of insecticidal of the potential of formulation combined with pheromone in field conditions

This second part of the work was carried out to study the feasibility of using pheromone combined with pseudo-stem as a baited trap, to optimize the efficacy of biopesticide in the field. It consisted of hacking one lure on a pseudo-stem trap and applying 30 g kaolin based formulation under the baited trap. The trial was conducted at an IITA-Cameroon experimental farm serving as a multiplication garden where numerous banana and

plantain cultivars were planted. Treatments were: i) pheromone baited trap without formulation (T0) and ii) pheromone baited trap with kaolin-based formulation (T1). Six traps were placed per field (500 – 1000 m^2) under the banana mat (3 traps of T0 and 3 traps of T1) and inspection was carried out once a week for 3 weeks. The traps were changed after two weeks (according to the observation of the later work, II.2.4.1). Alive, dead or mycosis insects were monitored around the banana mat, leaf sheath, soil near the mat, and mulch every 7 days.

II.3.6. Data analysis

II.3.6.1. Efficacy of fungi against banana weevil immature life stages

Data obtained from the experiment were statistically analyzed with R version 3.4.3. Cumulative mortality rates were corrected using Abbott's formula (Abbott, 1925). Arcsine or square root transformation was used to correct error distributions and normality of germination, corrected mortality and infection rates of adults, eggs, larvae, and pupae, as well as the percentage of the emerged larva and emerged adult of banana weevil were used as response variables in univariate 1-factor (isolate) ANOVA after arcsine or square root transformation of the response variables to correct for heteroscedasticity inherent in our type of data. Where significant factor F-tests ($P < 0.05$) were found, means were separated with Tukey Honestly Significant Difference (HSD) test at $\alpha = 0.05$ using R software version 3.4.3.

II.3.6.2. Data Analysis for epizootic potential of thermotolerant entomopathogenic isolates

The response variables mycelial growth, conidia production, and germination percentage of each of the six isolates across 7 constant temperatures were analyzed separately with a 2-factor analysis of variance using a complete randomized design. Where factor F-tests indicated significance ($P < 0.05$) means were compared with Tukey HSD. The response variables were log-transformed [Log (x+1)] where needed to correct the heterogeneity of the error variances inherent in the types of data presented.

In the horizontal transmission experiment, the effects of isolate and vector ratio on % infection and mortality were analyzed with 2-factor ANOVA using a complete randomized design. The values of the mortality and mycosis were arcsine-transformed to correct for the heterogeneity of error variances before their use in the ANOVA. Where factor F-test indicated

significance, means were separated with Tukey's HSD. The conidial yield of the four fungal isolates infecting banana weevil in the horizontal transmission tests was analyzed with 1-factor ANOVA using a complete randomized design. Means were separated with Tukey's HSD. ANOVAs and regressions were performed with R version 3.4.3.

II.3.6.3. Data analysis for the effect of environmental factors on fungal isolates

The number of colonies formed unit, colony diameter and conidia yield were log-transformed and analyzed using analysis of variance using R software version 3.4.3. The mean for each parameter was separated with Tukey at the threshold of 5 %. T-test was used to compare the two fungi isolates and the correlation of data between days' post-incubation.

II.3.6.4. Data analysis for the selection of ingredients for mycoinsecticide formulation

Data obtained from mass production, quality control was analyzed using analysis of variance through GLM procedure distribution binomial and distribution Poisson respectively for weevil mortality and conidia yield, and conidia viability.

The experiment for assessment of the compatibility of carriers with conidia had a factorial design with three main factors (fungal isolates with two levels, formulations with thirteen levels, and storage time with two levels) and four replicates. A three-way ANOVA on conidial viability data was performed. The data were transformed to Arcsine to meet the requirements of ANOVA for normal data distribution and homogeneity of variances (Mola et *al.*, 2012). The half-life (LT50) of germinated conidia was also determinate for each isolate.

Data from the evaluation of physico-chemical properties of carriers were subjected to analysis of variance followed by Tukey's test for multiple comparisons at significant level Pvalue = 0.05 (Traynham *et al.*, 2007).

II.3.6.5. Data analysis for the effect of formulations

II.3.6.5.1. Data analysis for the effect of formulations in the laboratory

This experiment had a factorial design with one main factor (formulations with ten levels) and four replicates. A one-way ANOVA on corrected mortality data was performed. The data were transformed to Arcsin to meet the requirements of ANOVA for normal data

distribution and homogeneity of variances (Mola *et al.*, 2012). The values of LT50 and LT90 were also determined for each formulation with a Probit analysis.

II.3.6.5.2. Data analysis for the effect of formulations in the greenhouse and field conditions

The growth parameters evaluated were analyzed with t student test. The number of leaves was analyzed with the Kruskal-Wallis test, a non-parametric test. The comparison of variance according to the treatments was done with a t-value test at a threshold of 5 %. The damage of interior and exterior of the rhizome, exterior, and interior of the base of the pseudo-stem, and damage of the periphery were transformed as $\text{Log}_{10}(n+1)$, to obtain the normal distribution and equal variances and analyzed statistically with ANOVA as the multifactorial analysis. The difference in the percentage of damage to varied parts of the plant was separated with student Tukey's test. The t-test was used to determine the level of difference in the pest damages between treatments for each part of the plant assessed. The logistic regression was used to determine the percentage of colonization, the number of living larva and/or dead. The turkey test at a threshold of 5 % served to evaluate the effect of treatments on the adults, larva, and pupa and mycoses eggs.

The general linear model procedure (GLM). was also used for the analysis of variance of treatments and means separated by the Student-Newman-Keuls test ($P = 0.05$). The analysis of banana weevil count was based on data transformed to log (weevil count + 1) and effect of *B. bassiana* on the weevil population presented as curves using regression analysis with GLM (Nankinga and Moore, 2000).

II.3.6.5.3. Data analysis for the effect of formulations in combination with pheromone

Mortality and mycosis rates were arcsin (sqrt (x)) transformed while the number of caught weevil were log (x+1) transformed. Data were submitted to one-way ANOVA after transformation. T-test was used to compare treatments while effect exposure time and interaction were separated using Tukey's test at level 5 %.

CHAPTER III. RESULTS AND DISCUSSION

III.1. Results

III.1.1. Characterization of fungal strains

III.1.1.1. Susceptibility of immature *C. sordidus* to entomopathogenic fungal isolates

III.1.1.2.1. Susceptibility of *C. sordidus* eggs and emerged larvae to entomopathogenic fungal isolates

While mycosis was recorded on both eggs and emerged larvae for all fungal isolates tested (Table III and fig.19). The entomopathogenic fungal isolates were significantly different regarding egg mycosis rates ($P = 0.002$), with only the three *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2) being significantly different from control, but not significantly different from *B. bassiana* isolates. Emerged larvae from treated eggs were horizontally infected by fungi even as larvae were transferred on untreated food. Isolates were significantly different in the horizontal infection of larvae ($P < 0.001$) with the three isolates of *M. anisopliae* being significantly different from control while no difference was found between *B. bassiana* isolates and the control. Combined mycosis was significantly higher for the three *M. anisopliae*'s isolates compared to the control ($P < 0.001$).

III.1.1.2.2. Susceptibility of *C. sordidus* 5th instar larvae to entomopathogenic fungal isolates

All isolates were pathogenic (fig.19 b and 19 f) to the 5th instar larvae of banana weevil *C. sordidus*, with significant high mortalities in the six isolates tested ($P < 0.001$). Overall, *M. anisopliae* isolates showed equal or superior virulence compared with *B. bassiana* isolates. The *B. bassiana*'s isolate BIITAC10.3.3 and *M. anisopliae* MIITAC11.3.4 were the most virulent isolates, causing 100.0 ± 0.00 % mortalities on treated larvae (Table V). The virulence in the other isolates was also high and ranged from 68.1 ± 9.21 to 95.8 ± 4.17 %. The values of LT_{50} and LT_{90} ranged from 3.02 to 7.76 days and 7.33 to 9.08 days respectively. The three *M. anisopliae* isolates and BIITAC10.3.3 had the shortest LT_{50} and LT_{90}. Results of mycosis tests ($P < 0.001$) (Table IV) revealed approximatively the same trend as mortality rates.

Table III. Pathogenicity of fungal isolates on eggs and emerged larva from treated eggs developing on the untreated substrate

Isolates	Eggs mycosis (%)	Emerged larva mycosis (%)	Combined mycosis (%)
		Beauveria bassiana	
BIITAC10.3.3	10.0 ± 3.10ab	50.0 ± 22.4abcd	26.0 ± 10.3bc
BIITAC6.2.2	6.00 ± 4.00ab	14.7 ± 9.04cd	12.0 ± 5.83c
BIITAC8.1.5	20.0 ± 10.5ab	33.3 ± 14.9bcd	30.0 ± 14.5bc
		Metarhizium anisopliae	
MIITAC11.3.4	32.0 ± 10.2a	100 ± 0.0a	96.0 ± 4.00a
MIITAC6.2.2	26.0 ± 2.44a	70.2 ± 12.5abc	68.0 ± 6.70ab
MIITAC6.4.2	36.0 ± 9.27a	80.0 ± 20.0ab	68.0 ± 16.9ab
Control	0b	0d	0c
	F = 4.70; df = 6; P = 0.002	F = 6.81; df = 6; P < 0.001	F = 13.15; df = 6; P < 0.001

*Larvae here were not treated but received the infection horizontally from eggs treated. Means followed by the same letter in the same column are not significantly different with the Tukey HSD test, $\alpha = 0.05$.

III.1.1.2.3. Susceptibility of *C. sordidus* pupae and emerged adult to entomopathogenic fungal isolates

When the banana weevil's pupae were treated with the different entomopathogenic isolates, pupal mortality and mycosis rate differed significantly between isolates (P = 0.002 and < 0.0001 respectively). The most virulent isolate was BIITAC6.2.2. BIITAC10.3.3 did not cause any mycosis, while pupal mycosis by other isolates was high and similar. Treated pupae transmitted the fungal inoculum to the emerging adults (fig. 19). The mortality of emerged adults were significantly high when pupae were treated with the three *M. anisopliae* isolates and *B. bassiana*'s isolate BIITAC8.1.5 (P = 0.002). Mycosis of subsequent adults was significantly high in the three *M. anisopliae* isolates (P = 0.002) (Table V). However, combined mortality and combined mycosis rates were high for all isolates except BIITAC10.3.3 (Table V).

Table IV. Pathogenicity and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates at 3.2×10^8 conidia/ml at against 5th instar larva of *Cosmopolites sordidus***,** 14 days' post-inoculation

Fungal Isolates	Mortality (%)	LT50(days) 95% FL	LT_{90} (days) 95% FL	Mycosis (%)
		Beauveria bassiana		
BIITAC10.3.3	100.00 ± 00a	4.19 (3.50-4.82)	9.08 (7.77-11.23)	60.00 ± 8.94b
BIITAC6.2.2	70.56 ± 11.17bc	7.76 (6.50-9.32)	*	80.55 ± 9.04ab
BIITAC8.1.5	68.05 ± 9.21c	6.07 (4.51-7.75)	*	75.00 ± 12.00ab
		Metarhizium anisopliae		
MIITAC11.3.4	100.00 ± 00a	4.10 (3.51-4.65)	7.33 (6.41-8.81)	100.00 ± 00a
MIITAC6.2.2	95.83 ± 4.17ab	4.03 (3.14-4.83)	8.96 (7.37-11.98)	78.33 ± 8.33ab
MIITAC6.4.2	94.44 ± 5.55ab	3.02 (1.88-3.95)	7.38 (5.66-11.50)	68.17 ± 4.17b
	F = 5.85; df = 5; P <0.0007			F = 3.87; df = 5; P < 0.0008

* mortality less than 90%.

Mean mortality and mycosis followed by the same lower-case letters are not significantly different by Tukey's HSD at $P > 0.05$. Lethal time 50 (LT_{50}) and 90 (LT_{90}) (days) at 95% fiducial limit (FL)

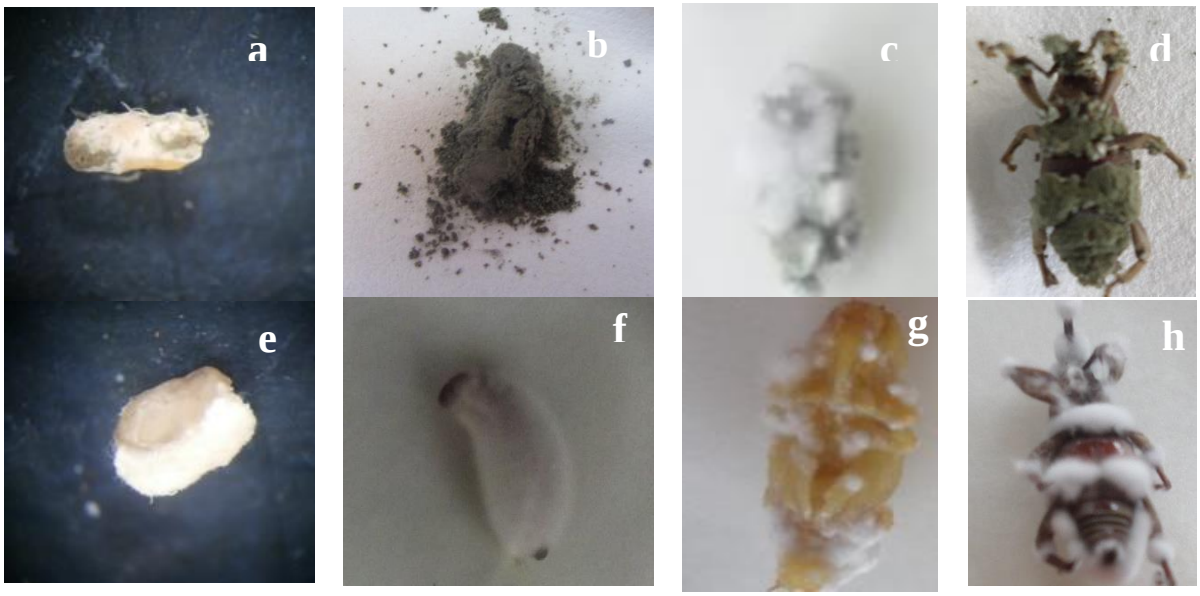

Fig. 19. Egg (a,e), larva (b, f), pupa (c, g) and adult (d, h) respectively infected by *M. anisopliae* and *B. bassiana*.

III.1.1.2. Endophytic colonization

None of the fungal isolates tested in this study were able to colonize leaves and corm of the cultivar "Ebang" through the three methods used. Entomopathogenic fungi were not re-isolated from all treated plants, neither from any of the control plants. No leaves or corm pieces were colonized after 20 days of incubation. No larva was found dead with fungi outgrowth when the leaves extract of treated plants was added to larva food.

III.1.2. Epizootic potential of thermotolerant fungal isolates

III.1.2.1. Effect of temperature conditions on conidia viability

Conidia germination rate (Table VI) was highly affected by temperature ($F = 787.7$; $df = 6, 209$; $P < 0.001$) and isolates ($F = 67.4$; $df = 5, 209$; $P < 0.001$), but the effect of temperature on conidia germination was not similar for all isolates (temperature x isolate: $F = 34.4$ $df = 30, 209$; $P < 0.001$).

Table V. Mortality and mycosis rates (Mean ± SE) of pupae and freshly emerged adults from pupa treated with *Beauveria bassiana* and *Metarhizium anisopliae* isolates

Isolates	Pupal mortality (%)	Pupal mycosis (%)	Emerged adult mortality* (%)	Infected adults (%)	Combined mortality (%)	Combined mycosis (%)
Beauveria bassiana						
BIITAC10.3.3	18.25 ± 7.08b	0.00 ± 0.00b	27.74 ± 9.93c	12.50 ± 12.50bc	39.30 ± 10.21b	12.50 ± 12.50b
BIITAC6.2.2	73.41 ± 12.30a	75.00 ± 9.37a	38.90 ± 20.03bc	45.83 ± 20.83ab	95.24 ± 4.76a	79.44 ± 7.22a
BIITAC8.1.5	45.33 ± 9.63ab	49.67 ± 13.75a	86.67 ± 8.16ab	54.00 ± 12.88ab	93.81 ± 3.81a	51.52 ± 8.90a
Metarhizium anisopliae						
MIITAC11.3.4	37.73 ± 8.87b	78.33 ± 9.80a	100.00 ± 0.00a	85.83 ± 6.88a	100 ± 00a	77.18 ± 2.95a
MIITAC6.2.2	38.45 ± 8.60ab	86.11 ± 10.90a	90.83 ± 5.83a	66.67 ± 11.38a	94.76 ± 3.33a	68.94 ± 8.86a
MIITAC6.4.2	41.90 ± 6.14ab	73.61 ± 10.20a	90.74 ± 9.26a	88.90 ± 7.03a	94.44 ± 2.78a	77.85 ± 5.96a
	F = 4.88; df = 5; P < 0.002	F = 9.64; df = 5; P < 0.001	F = 7.32; df = 5; P < 0.001	F = 5.07; df = 5; P < 0.002	F = 13.1; df = 5; P < 0.001	F = 19.8; df = 5; P < 0.001

*Adults were horizontally infected as a result of pupal artificial infection. Means followed by the same letter in the same column are not significantly different with Tukey's HSD test, P > 5%.

Conidia germinated at all temperatures except at 37 °C. Optimum germination rates of *B. bassiana* BIITAC8.1.5 were highest at 20 and 25 °C (70.2 ± 16.4 and 92.5 ± 0.80 % respectively), while conidia germination of the isolates BIITAC10.3.3 and BIITAC6.2.2 were highest but not significantly different at 20, 25 and 29 °C (Table VI). A wider range of high germination rates was obtained for the three *M. anisopliae* isolates from 20 to 33 °C (Table VI). Conidia germination rates of all fungus isolates were less than 11 % at 13 °C. When incubated at 15 °C**,** the germination rates were significantly high for *B. bassiana* BIITAC8.1.5 ($P < 0.001$). The germination rates of all isolates were relatively high (90.9 ± 0.28 and 94.7 ± 0.36 %) at 20 °C, except BIITAC6.2.2 and MIITAC6.2.2 for which conidia was less than 72 % (Table VI). At 25 °C**,** MIITAC6.2.2 and MIITAC11.3.4 showed significantly high germination rate ($P < 0.001$) followed by MIITAC6.4.2 which was not different from BIITAC6.2.2 and BIITAC10.3.3, compared with BIITAC8.1.5. When the isolates were incubated at 29 and 33 °C, high germination rates were obtained for the three *M. anisopliae* isolates, followed by *B. bassiana* BIITAC6.2.2 and BIITAC10.3.3 ($P < 0.001$). BIITAC8.1.5 had the lowest germination rate at both 29 and 33 °C (11.8 ± 1.52 and 9.50 ± 1.05 % respectively) compared with all the others.

Table VI. Effect of seven constant temperatures on conidial viability (mean % viability ± SE) of six entomopathogenic fungi isolates

Temperature (°C)	BIITAC10.3.3	BIITAC6.2.2	BIITAC8.1.5	MIITAC11.3.4	MIITAC6.2.2	MIITAC6.4.2
13	10.0 ± 1.15bA	6.05 ± 1.70dA	7.55 ± 0.95bcA	0cB	4.70 ± 2.03cA	5.10 ± 2.60 bcA
15	12.6 ± 0.48bBC	6.55 ± 0.77dD	24.4 ± 1.61bA	2.85 ± 1.16bcE	7.65 ± 0.62bcCD	13.6 ± 1.34bB
20	92.7 ± 0.54aA	68.5 ± 1.70bB	92.5 ± 0.80 aA	90.9 ± 0.28abcA	71.5 ± 3.71abcB	94.7 ± 0.36aA
25	76.9 ± 10.10aAB	88.0 ± 0.69aAB	70.2 ± 16.4aB	99.6 ± 0.13aA	99.4 ± 1.17abA	98.1 ± 0.57aAB
29	78.4 ± 5.72aB	82.0 ± 3.67abB	11.8 ± 1.52bC	99.4 ± 0.15abA	99.7 ± 0.24abA	99.7 ± 0.12aA
33	24.9 ± 2.00bB	42.7 ± 9.56cB	9.50 ± 1.05bcC	99.6 ± 2.15aA	100 ± 0.00aA	99.4 ± 0.36aA
37	0c	0e	0c	0 ± 0c	0c	0c
	F = 82.7; df=6; P < 0.001	F = 115.5; P < 0.001	F = 36.12; P < 0.001	F = 1196; P < 0.001	F = 727.8; P < 0.001	F = 262.9; P < 0.001

Means followed by the same lower (upper) case letter within a column (row) are not significantly different with Tukey HSD test, $\alpha = 5\%$ for temperatures (isolates)

III.1.2.2. Effect of temperature conditions on mycelial growth

Similar to conidia germination (i.e., viability), mycelia growth (fig. 20) was strongly affected by temperature ($P < 0.001$) and isolate $F = 15.1$; $df = 5, 188$; $P < 0.001$), but the effect of temperature on mycelia growth was not the same for the isolates as evident in the interaction term ($F = 8.82$; $df = 30, 188$; $P < 0.001$). The *B. bassiana* isolates, BIITAC8.1.5 produced the largest colonies at 20 °C (61.20 ± 8.40 mm) with similar levels of mycelia growth at 15, 25 and 29 °C.

The *Metarhizium* isolate MIITAC11.3.4 showed optimum mycelia growth between 20 and 29 °C with similar growth within this range (fig. 20). MIITAC6.4.2 produced the high mycelial growth at 25 °C (60.3 ± 6.12 mm), which was not significantly different at 15, 20, and 29°C. Mycelia growth was high for MIITAC6.2.2 at 13-29 °C with no significant difference at 15 and 33 °C (Fig. 20). The isolates BIITAC8.1.5, MIITAC6.4.2, BIITAC10.3.3, and BIITAC6.2.2 showed significantly high colony growth when incubated at 13 °C ($P = 0.003$). When the isolates were incubated at 15 and 20 °C, *B. bassiana* BIITAC8.1.5 produced the highest colonies. At 15 °C, the colony size of the isolate BIITAC8.1.5 was followed by MIITAC6.4.2, MIITAC6.2.2, and BIITAC10.3.3 (which had the same trends) whereas at 20 °C, the same isolate was followed by BIITAC6.2.2, MIITAC6.2.2, and MIITAC6.4.2. Mycelia growth from temperature 25°C was significantly between isolates ($P = 0.002$), with the highest growth for MIITAC6.4.2, BIITAC6.2.2, and BIITAC8.1.5 which were not different within them. At 29 °C, fungi isolates showed similar growth while at 33 °C the three *M. anisopliae* isolates produced higher mycelial growth ($P < 0.001$) compared to *B. bassiana* isolates among which not significant difference was found.

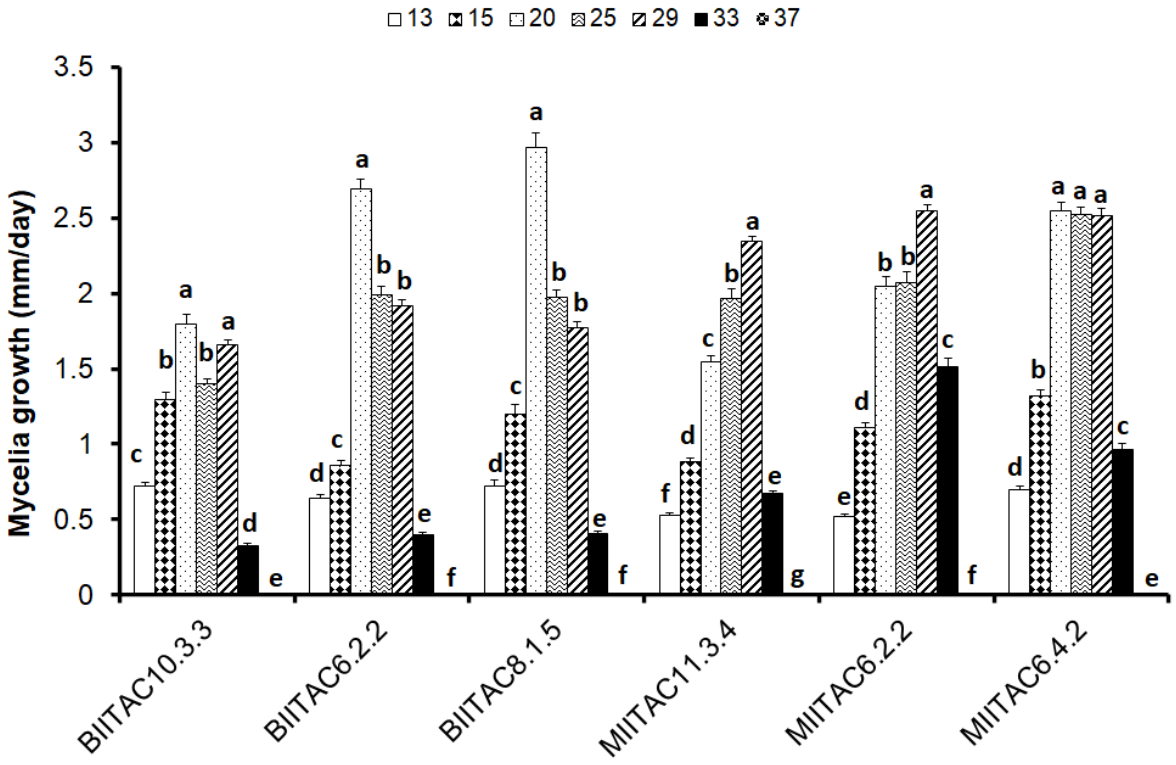

Fig. 20. Mycelial growth of six entomopathogenic fungal isolates incubated on PDA medium at seven constant temperatures (13, 15, 20, 25, 29, 33, and 37 ℃).

III.1.2.3. Effect of temperature conditions on conidiation

Total conidia production by the six tested fungi was highly significantly affected by temperatures ($P < 0.001$) and isolates ($P < 0.001$), but relationship between conidia production of the isolates did not follow similar trends across temperatures (interaction of temperature and isolate: $F = 92.4$; $df = 30, 167$; $P < 0.001$). All three *B. bassiana*'s isolates and one *M. anisopliae* isolate - MIITAC6.2.2 – produced conidia in the range of 13 – 33 °C compared with *M. anisopliae's* isolates MIITAC11.3.4 and MIITAC6.4.2 which respectively produced conidia in the range of 13 – 29 °C and 20 – 29 °C (Table VII). The widest optimum range of temperature for the highest conidia production was at 13 - 29 °C for MIITAC6.2.2, followed by BIITAC10.3.3 (13 - 25 °C) and MIITAC11.3.4 and BIITAC8.1.5 which had both highest conidia production at 15 - 25 °C. The isolates BIITAC6.2.2, MIITAC11.3.4, and BIITAC8.1.5, and MIITAC6.4.2 showed a narrow optimum temperature range for high conidia yield, 13and 25 °C respectively (Table VII).

When the six isolates were incubated at 13 °C, BIITAC6.2.2 ($7.50 \pm 3.30 \times 10^9$ conidia /ml) produced a significant high conidia amount compare to other isolates ($P < 0.001$). At 15 °C, the conidia production was significantly high for all isolates studied except MIITAC6.4.2 which did not produce conidia at this temperature (Table VII). The highest level of conidia production at 20 °C was for BIITAC6.2.2 and BIITAC10.3.3 ($1.88 \pm 0.6 \times 10^9$ and $2.01 \pm 0.56 \times 10^9$ conidia/ml respectively). At 25 °C, the three *Beauveria* isolates produced the highest conidia yield, while at 29 °C all the fungal isolates produced a similar quantity of conidia ($1.55 \pm 0.42 \times 10^7$ -$1.73 \pm 0.17 \times 10^8$ conidia/ml) with exception of MIITAC6.4.2 which was low ($1.43 \pm 0.45 \times 10^5$ conidia/ml). At 33 °C MIITAC6.2.2 had the highest conidia production followed by the three *B. bassiana's* isolates. Conidia production was low for MIITAC6.4.2 at all temperatures. None of the isolates produced any conidia at 37 °C (Table VII).

Table VII. Conidia production of entomopathogenic fungi isolates at seven constant temperatures

Temp (°C)	BIITAC10.3.3	BIITAC6.2.2	BIITAC8.1.5	MIITAC11.3.4	MIITAC6.2.2	MIITAC6.4.2
13	$(6.00 \pm 3.10) \times 10^8$aB	$(7.50 \pm 3.30) \times 10^9$aA	$(2.50 \pm 0.64) \times 10^8$bB	$(3.52 \pm 2.02) \times 10^7$cC	$(4.35 \pm 0.30) \times 10^7$abBC	0 dD
15	$(8.61 \pm 1.56) \times 10^8$aA	$(1.62 \pm 0.23) \times 10^9$bA	$(5.43 \pm 0.55) \times 10^8$abA	$(4.10 \pm 0.51) \times 10^8$aA	$(4.93 \pm 0.56) \times 10^7$abA	0 dB
20	$(2.01 \pm 0.56) \times 10^9$aA	$(1.88 \pm 0.60) \times 10^9$bA	$(4.45 \pm 2.36) \times 10^8$abB	$(2.83 \pm 1.11) \times 10^8$aB	$(3.35 \pm 0.63) \times 10^8$aB	$(8.18 \pm 1.20) \times 10^5$bC
25	$(2.02 \pm 0.80) \times 10^9$aA	$(2.12 \pm 0.65) \times 10^9$bA	$(2.16 \pm 1.10) \times 10^9$aA	$(2.02 \pm 0.70) \times 10^8$abB	$(1.64 \pm 0.32) \times 10^8$abB	$(6.90 \pm 0.22) \times 10^6$aC
29	$(7.54 \pm 5.53) \times 10^7$bA	$(1.73 \pm 0.17) \times 10^8$cA	$(1.55 \pm 0.42) \times 10^7$cA	$(3.57 \pm 1.40) \times 10^7$bcA	$(1.33 \pm 0.23) \times 10^8$abA	$(1.43 \pm 0.45) \times 10^5$cB
33	$(2.20 \pm 0.34) \times 10^6$bB	$(8.87 \pm 1.80) \times 10^5$dB	$(2.85 \pm 0.53) \times 10^6$dB	0dC	$(6.60 \pm 2.27) \times 10^7$bA	0dC
37	0c	0e	0d	0d	0c	0d

Temp: temperature.

Means followed by the same lower (upper) case letter within the column (row) are not significantly different with the Tukey HSD test, $\alpha = 5\%$ for temperatures (isolates).

III.1.2.4. Horizontal transmission potential of thermotolerant isolates against adult banana weevil

Mortality (fig. 21A) and infection (fig. 21B) rates of non-inoculated weevils were higher for BIITAC6.2.2 than all the other five isolates, while the same responses for the three other isolates were not different from the control ($P < 0.001$ and $P = 0.03$ for mortality and infection respectively). All isolates showed the ability of auto-disseminate from inoculated to non-inoculated insects, but transmission potential varied with vector ratio.

When 10, 30 and 50 % of adult weevils were treated with a fungus isolate, mortality of non-inoculated weevils was higher than in the control ($F = 3.75$; df = 4, 19; $P = 0.026$; $F = 11.1$; df = 4, 19; $P < 0.001$ and $F = 10$; df = 4, 19; $P < 0.001$ respectively for 10, 30, and 50 % vector ratios). BIITAC6.2.2 caused higher mortality than control at all vector ratios (fig. 21A). No difference in infection rates of non-inoculated insects at vector ratio of 10 % ($F = 1.57$; df = 4, 19; $P = 0.23$) and 30 % ($F = 1.32$; df = 4, 19; $P = 0.31$) for this isolate (BIITAC6.2.2). When 50 % adult weevils were treated, infection rate of BIITAC6.2.2 was also significantly higher than that of control ($F = 6.25$; df = 4, 19; $P = 0.003$). Mean mortality of control insects was below 1 % (0.83 %) and none of the dead insects showed mycosis (fig 21A and 21B). BIITAC6.2.2 only caused 50 % mortality of non-inoculated weevils at vector ratio 50 %.

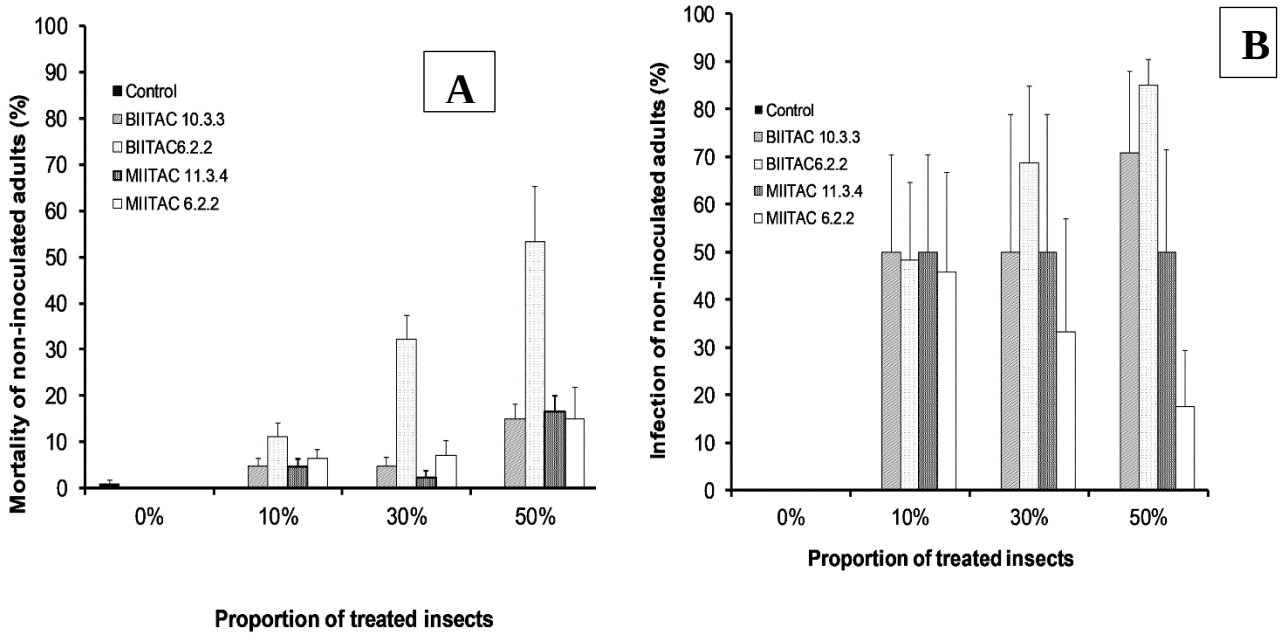

Fig. 21. Percent mortality (A) and mycosis (B) (mean + SE) in experiments of horizontal transmission potential of four entomopathogenic fungal isolates (*B. bassiana* isolates BIITAC10.3.3, BIITAC6.2.2 and *M. anisopliae* isolates MIITAC11.3.4, MIITAC6.2.2) with four increasing levels of vector (inoculated weevils) ratios.

The mean conidia yield by non-inoculated insects was higher for isolate BIITAC6.2.2, $(3.43 \pm 1.90) \times 10^{10}$ conidia/mL compared with the other isolates ($F = 59.7$; $df = 3, 39$; $P < 0.001$) while BIITAC10.3.3 and MIITAC11.3.4 produced similar numbers of conidia ($1.08 \pm 0.05 \times 10^{10}$ and $7.80 \pm 2.58 \times 10^{9}$ conidia/ml respectively). MIITAC6.2.2 produced the lowest number of conidia ($4.70 \pm 1.61 \times 10^{8}$ conidia/ml) compared with the four isolates used in this study (fig. 22).

When 10, 30 and 50 % of adult weevils were treated with a fungus isolate, mortality of non-inoculated weevils was higher than in the control ($F = 3.75$; $df = 4, 19$; $P = 0.026$; $F = 11.1$; $df = 4, 19$; $P < 0.001$ and $F = 10$; $df = 4, 19$; $P < 0.001$ respectively for 10, 30, and 50 % vector ratios). BIITAC6.2.2 caused higher mortality than control at all vector ratios (fig. 21A). No difference in infection rates of non-inoculated insects at vector ratio of 10 % ($F = 1.57$; $df = 4, 19$; $P = 0.23$) and 30 % ($F = 1.32$; $df = 4, 19$; $P = 0.31$) for this isolate (BIITAC6.2.2). When 50 % adult weevils were treated, infection rate of BIITAC6.2.2 was also significantly higher than that of control ($F = 6.25$; $df = 4, 19$; $P = 0.003$). Mean mortality of control insects was below 1 % (0.83 %) and none of the dead insects showed mycosis (fig 21A and 21B). BIITAC6.2.2 only caused 50 % mortality of non-inoculated weevils at vector ratio 50 %.

The mean conidia yield by non-inoculated insects was higher for isolate BIITAC6.2.2, $(3.43 \pm 1.90) \times 10^{10}$ conidia/mL compared with the other isolates ($F = 59.7$; $df = 3, 39$; $P < 0.001$) while BIITAC10.3.3 and MIITAC11.3.4 produced similar numbers of conidia ($1.08 \pm 0.05 \times 10^{10}$ and $7.80 \pm 2.58 \times 10^{9}$ conidia/ml respectively). MIITAC6.2.2 produced the lowest number of conidia ($4.70 \pm 1.61 \times 10^{8}$ conidia/ml) compared with the four isolates used in this study (fig. 22).

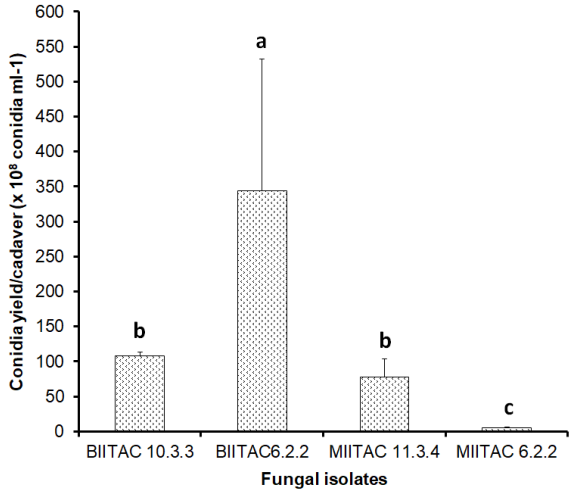

Fig. 22. Fungal conidia yield (mean ± SE) by insect's cadaver for four fungal isolates (BIITAC10.3.3, BIITAC6.2.2, MIITAC11.3.4 MIITAC6.2.2). Means followed by the same letter are not significantly different (Tukey HSD, $\alpha = 0.05$).

III.1.3. Effect of some environmental factor on the two suitable strains

III.1.3.1. pH toxicity on entomopathogenic fungi growth

III.1.3.1.1. pH effect on conidia germination

The results of conidia germination revealed a positive correlation between data of 4 days' post inoculation (DPI) and 10 DPI ($t = 17.44$; $df = 110$; $P < 0.0001$; $cor = 0.82$) meaning that when days of incubation increase, the number of colonies formed unit increase. At the fourth day post inoculation, The colony formed unit was ranked 0 - 3420 cfu and 0 - 2170 cfu for BIITAC6.2.2 and MIITAC11.3.4 respectively. Colonies were formed at pH range 3 - 13 and 3 - 12 for BIITAC6.2.2 and MIITAC11.3.4 respectively. The colony formed units were significant affected by isolates ($t = 3.44$; $df = 110$; $P < 0.0001$), pH values ($F = 2211.3$; $df = 13$; $P < 0.0001$) and interaction isolates-pH values ($F = 179.6$; $df = 13$; $P < 0.0001$).

The optimum pH range for colony formation was 4 - 12 for both isolates. The isolate BIITAC6.2.2 showed significant high cfu at pH 6 which were not significantly different between them. Whereas, the pH value 5 highly favored colonies formation of MIITAC11.3.4, but the number of colonies was similar from pH values 4, 6, 7, 8, 9, 10, and 12. (Table VIII).

When the incubation period was extended for 6 more days (on day 10), the pH tolerance range increased at 3 - 13.5 for both isolates. Colonies appeared at pH 13.5 (2500 ±

130 cfu) and increase at pH 13 (2300 ± 180 cfu) for BIITAC6.2.2 compare to day 4. For MIITAC11.3.4, colonies were formed at 13 (1370 ± 380 cfu) and 13.5 (810 ± 40 cfu). At 10 DPI, isolates ($t = 4.54$; $df = 110$; $P < 0.0001$), pH levels ($F = 1202.7$; $df = 13$; $P < 0.0001$) and interaction pH -isolate ($F = 3.86$; $df = 13$; $P < 0.0001$) affected significantly number of colonies formed unit. The pH optimum ranges for colony formation were 4 - 13.5 and 3 - 13 for BIITAC6.2.2 and MIITAC11.3.4 respectively (Table VIII).

Table VIII. Conidia germination based on the number of colony form unit four days and ten days' post-inoculation

PH values	BIITAC6.2.2		MIITAC11.3.4	
	4dpi	10dpi	4dpi	10dpi
2	00 ± 00 d	00 ± 00 c	00 ± 00 c	00 ± 00 d
3	(2.05 ± 0.02) x10^3 bc	(2.05 ± 0.02) x10^3 b	(1.30 ± 0.26) x10^3 b	(1.30 ± 0.26) x10^3 abc
4	(2.62 ± 0.21) x10^3 ab	(2.62 ± 0.21) x10^3 ab	(1.87 ± 0.10) x10^3 ab	(1.87 ± 0.10) x10^3 a
5	(2.57 ± 0.76) x10^3 ab	(2.57 ± 0.76) x10^3 ab	(2.17 ± 0.02) x10^3 a	(2.17 ± 0.02) x10^3 a
6	(3.42 ± 0.15) x10^3 a	(3.42 ± 0.15) x10^3 a	(1.92 ± 0.23) x10^3 ab	(1.92 ± 0.23) x10^3 a
7	(2.77 ± 0.23) x10^3 ab	(2.77 ± 0.23) x10^3 ab	(1.78 ± 0.05) x10^3 ab	(1.78 ± 0.05) x10^3 ab
8	(2. 30 ± 0.09) x10^3 ab	(2. 30 ± 0.09) x10^3 ab	(1.70 ± 0.30) x10^3 ab	(1.70 ± 0.30) x10^3 ab
9	(2.15 ± 0.23) x10^3 ab	(2.15 ± 0.23) x10^3 ab	(1.70 ± 0.24) x10^3 ab	(1.70 ± 0.24) x10^3 ab
10	(2.20 ± 0.11) x10^3 abc	(2.20 ± 0.11) x10^3 ab	(1.80 ± 0.20) x10^3 ab	(1.80 ± 0.20) x10^3 ab
11	(2.67 ± 0.17) x10^3 ab	(2.67 ± 0.17) x10^3 ab	(1.72 ± 0.10) x10^3 ab	(1.72 ± 0.10) x10^3 ab
12	(2.30 ± 0.24) x10^3 abc	(2.30 ± 0.24) x10^3 ab	(1.37 ± 0.12) x10^3 ab	(1.37 ± 0.12) x10^3 abc
13	(1.40 ± 0.21) x10^3 c	(2.30± 0.18) x10^3 ab	00 ± 00 c	(1.37 ± 0.38) x10^3 abc
13.5	00 ± 00 d	(2.50± 0.13) x10^3 ab	00 ± 00 c	(0.81 ± 0.04) x10^3 c
14	00 ± 00 d	00 ± 00 c	00 ± 00 c	00 ± 00 d
	$F = 1262.2$; $df = 13$; $P < 0.001$	$F = 951.7$; $df = 13$; $P < 0.001$	$F = 427.1$; $df = 13$; $P < 0.001$	$F = 1191$; $df = 13$; $P < 0.001$

dpi= days post inoculations.
Means followed by the same letter in the same column are not significantly different with the Tukeys HSD test, $\alpha = 5\%$.

III.1.3.1.2. pH effect on mycelial growth

The mycelia growth vary slightly between isolates ($t = -2.30$; $df = 1566$; $P = 0.02$) after 15 days' post inoculation. The pH levels significantly affected on fungi growth ($F = 632.6$; $df = 13$; $P < 0.0001$) (fig. 23). The mycelia growth of isolate BIITAC6.2.2 was between of 0 ±

00 mm/day and 3.30 ± 0.05 mm/day and tolerated pH 4 - 13 with significant highest growth at pH values 12 (3.30 ± 0.05 mm/day). However, no difference was found between pH 4 (2.78 ± 0.07 mm/day) to 11 (3.15 ± 0.05 mm/day). The isolate MIITAC11.3.4 was ranked 0 - 3.37 ± 0.12 mm/day (fig. 23) with highest length at pH value 10 (3.37 ± 0.12 mm/day) followed by pH 9 and pH 11 which had similar trend. The pH range tolerance was 3 - 13 for MIITAC11.3.4. PDA media at pH 2, 13.5 and 14 (fig. 23) was toxic for both isolates, added with pH 3 which was also toxic to BIITAC6.2.2. The optimum range was at pH 4 - 12 and 9 - 11 for BIITAC6.2.2 and MIITAC11.3.4 respectively.

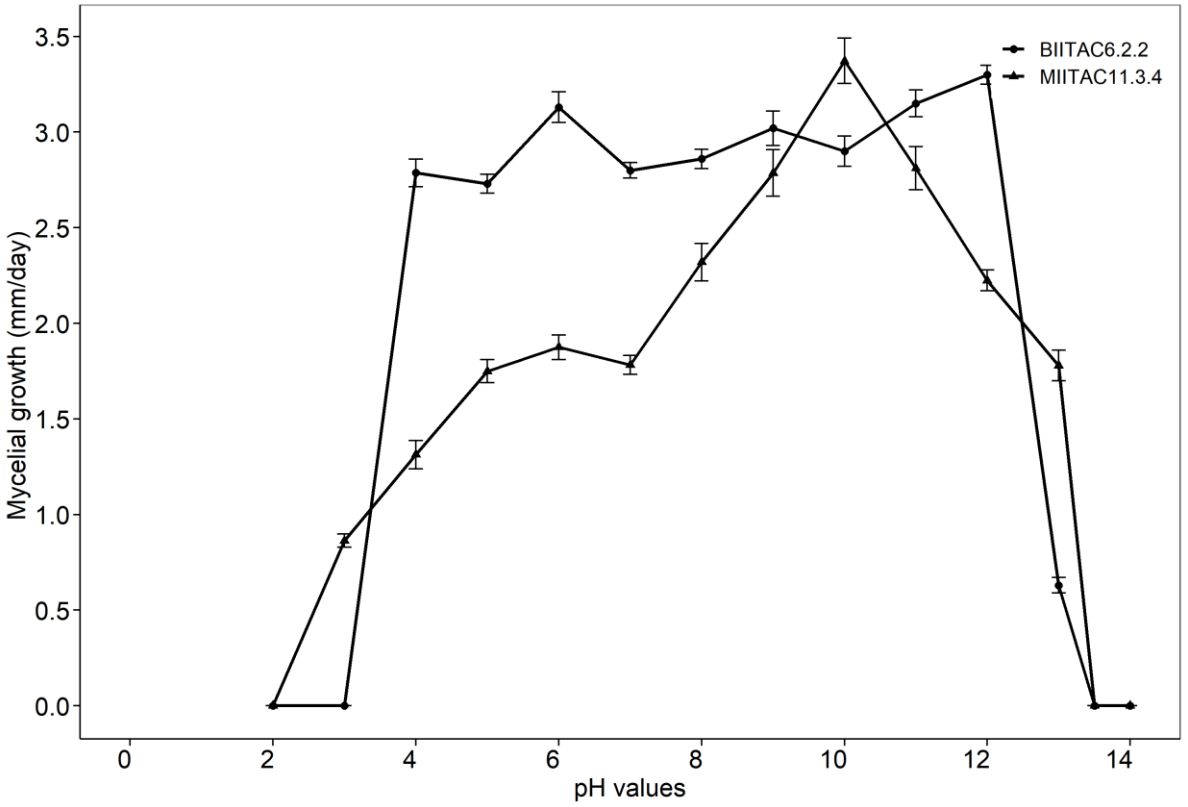

Fig. 23. Mycelial growth of fungal isolates at different pH value of PDA media 15 days post-incubation.

III.1.3.1.3. pH effect on conidia yield

When conidia were produced on PDA media at different pH, the conidia production was significant different between isolates ($t = 3.23$; $df = 110$; $P < 0.0001$) with high conidia production for BIITAC6.2.2. Our study also showed significant difference in conidia

production between pH (F = 633.9; df = 13; P < 0.0001) and in interaction pH – isolate (F = 6.65; df = 13; P < 0.0001). Beauveria BIITAC6.2.2 showed significant high conidia production at pH 7 (1.25 ± 0.36 x 10^8 conidia/mL) and pH 8 which was not significantly different at pH 4, 5, 6,9, 10, 11, 12 and 13. Metarhizium MIITAC11.3.4 showed high conidia production at pH 4 (1.25 ± 0.15 x 10^8 conidia/mL) and 5 (9.32 ± 1.87 x 10^7 conidia/mL), which was not significantly different with conidia production at pH values 6, 9, 10 and 11 (1.25 ± 0.15 x 10^8 conidia/mL). No conidia were produced at pH 2, 13.5 and 14 for both isolates. The pH tolerance range for conidiogenesis was 3 - 13 for both isolates but their optimum ranges were different 3 - 13 and 4 - 11 for BIITAC6.2.2 and MIITAC11.3.4 respectively (Table IX).

Table IX. Conidia production of fungal isolates at different pH levels of PDA media 15 days post-incubation

pH values	BIITAC6.2.3	MIITAC11.3.5
2	00 ± 00 c	00 ± 00 e
3	(1.00 ± 0.09) x10^7 b	(3.14 ± 2.04) x10^7 c
4	(1.25 ± 0.36) x10^8 ab	(1.25 ± 0.15) x10^8 a
5	(1.72 ± 0.6) x10^8 ab	(9.32 ± 1.90) x10^7 a
6	(1.42 ± 0.76) x10^8 ab	(8.00 ± 2.70) x10^7 ab
7	(2.40 ± 0.40) x10^8 a	(2.33 ± 0.53) x10^7 bc
8	(2. 45 ± 0.16) x10^8 a	(4.20 ± 1.23) x10^7 abc
9	(5.53 ± 2.70) x10^7 ab	(8.80 ± 0.71) x10^7 ab
10	(8.81 ± 3.70) x10^7 ab	(5.96 ± 1.04) x10^7 abc
11	(1.42 ± 0.46) x10^8 ab	(5.87 ± 0.94) x10^7 abc
12	(6.34 ± 1.25) x10^7 ab	(6.25 ± 1.25) x10^5 d
13	(2.44± 0.62) x10^7 ab	(1.87 ± 0.41) x10^5 d
13.5	00 ± 00 c	00 ± 00 e
14	00 ± 00 c	00 ± 00 e
	df= 13; F= 217.7; P < 0.001	df= 13; F= 716.4; P < 0.001

Means followed by the same letter in the same column are not significantly different with Tukeys HSD test, α = 5%.

III.1.3.2. Effect of photoperiod on entomopathogenic fungi growth

III.1.3.2.1. Effect of white light regimes on EPF germination

Beauveria bassiana BIITAC6.2.2 and Metarhizium MIITAC11.3.4 conidia germinated at all photoperiod conditions tested (Table X). Whereas response between isolates had less effect on the number of colonies formed unit ($t = 2.13$; $df = 22$; $P = 0.04$). No significant difference was observed between photoperiod ($F = 0.85$; $df = 2$; $P = 0.44$) while the number of cfu varied significantly in the interaction photoperiod-isolate ($F = 25.26$; $df = 2$; $P < 0.0001$). The colony formed units of MIITAC11.3.4 varied between photo period conditions ($F = 4.58$; $df = 2$; $P < 0.001$) with highest cfu in continuous light (2700 ± 15.00 cfu). For BIITAC6.2.2, 1990 – 3050 cfu (Table X) were counted and statistical analysis revealed no significant difference between photoperiod conditions ($F = 40.70$; $df = 2$; $P < 0.001$).

Table X. Conidia viability of fungi strains four days' post inoculation under different photoperiod

Photoperiod	BIITAC6.2.2	MIITAC11.3.4
12light:12dark	$(2.90 \pm 0.18) \times 10^3$a	$(1.88 \pm 0.05) \times 10^3$ b
continuous dark	$(3.05 \pm 0.22) \times 10^3$a	$(2.02 \pm 0.06) \times 10^3$b
continuous light	$(1.99 \pm 0.17) \times 10^3$b	$(2.70 \pm 0.15) \times 10^3$a
	F= 40.70; df= 2; P < 0.001	F= 22.18; df= 2;P < 0.001

Means followed by the same letter in the same column are not significantly different with the Tukeys HSD test, $\alpha = 5\%$.

III.1.3.2.2 Effect of white light regime on mycelia growth of EPF

All isolates, BIITAC6.2.2 and MIITAC11.3.4 grew at tested photoperiod, and there was no difference between isolates ($t = 11.14$; $df=334$; $P < 0.001$). The mycelial growth was highly significantly affected by photoperiod ($F = 20.70$; $df = 2$;$P < 0.001$) and interaction photoperiod-isolate ($F = 15.44$; $df =2$; $P < 0.001$) at 15 days' post inoculation. BIITAC6.2.2 growth was 2.08 ± 0.02 – 2.45 ± 0.07 mm/day with highest growth when petri dishes were incubated at photoperiod 12L:12D and continuous dark, which showed similar mycelia growth (fig. 24) compare to continuous light ($F = 15.77$; $df = 2$; $P < 0.001$), MIITAC11.3.4

growth was significantly different between the three photoperiod conditions (F = 18.61; df = 2; P < 0.001), ranked 1.37 ± 0.04 – 2.07 ± 0.07 mm/day with growth at photoperiod 12L:12D (fig. 24).

Different color of fungi colony was observed when fungi isolates were incubated at different photoperiod. For *B. bassiana* BIITAC6.2.2 colony pigmentation was yellow, pale yellowish, and white cream at 24 L:0 D, 12 L:12 D, and 0 L:24 D respectively. Color of MIITAC11.3.4 colonies also varied according to photoperiod, olive green, green, and grey-green at continuous light, 12 L:12 D, and continuous dark respectively.

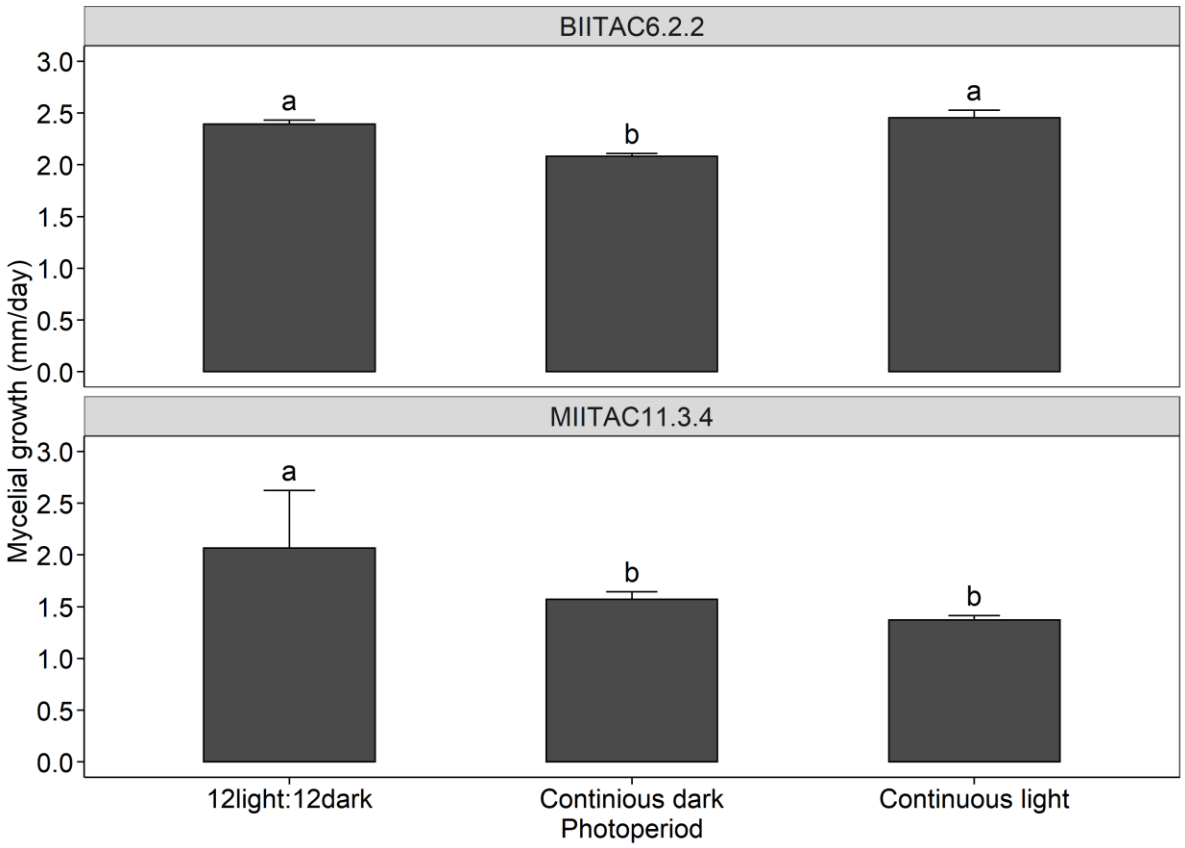

Fig. 24 : Mycelial growth of fungal isolates at different photoperiods on PDA media 15 days post-incubation.

III.1.3.2.3 Effect of white light regimes on EPF conidia yield

Results of conidia production on PDA media under different photoperiod, significant different between isolates (t = 8.80; df = 22; P < 0.001). The photoperiod (F =4.60; df = 2; P

< 0.02) also affected conidia production but slightly. Interaction photoperiod – isolate was not significantly different (F = 0.48; df = 2; P = 0.62). Conidia production of Beauveria BIITAC6.2.2 was similar under continuous dark, continuous light and 12 light :12 dark respectively, 4.4 x 10^8 conidia/mL, 4.87 x 10^8 conidia/mL and 3.97 x 10^8 conidia/mL (fig. 25). For Metarhizium MIITAC11.3.4, conidia reproductions were between (1.41 x 10^7 – 1.80 x 10^8 conidia/mL) with high conidia production under 12 light :12 dark condition followed by continuous dark (fig. 25).

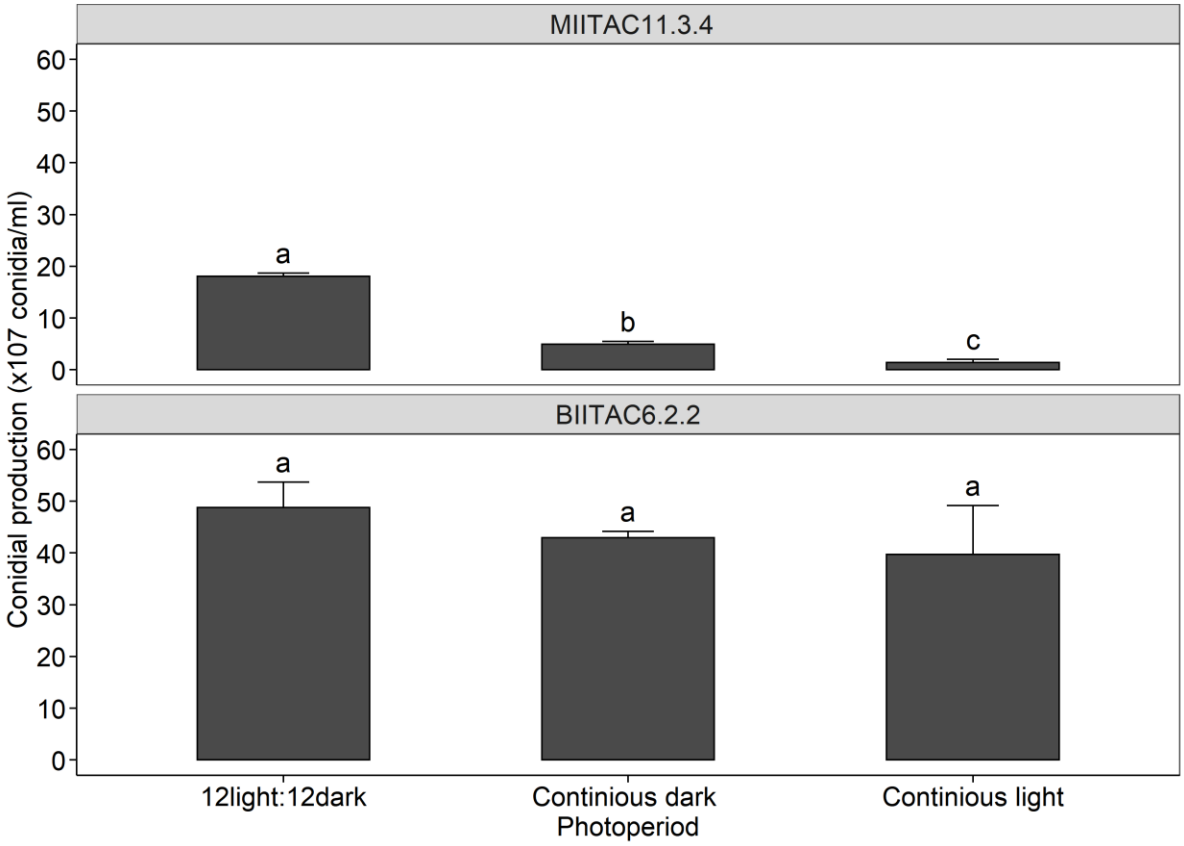

Fig. 25. Conidia yield of fungal isolates at different photoperiod after 15 days of incubation.

III.1.3.3. Effect of UVB light on entomopathogenic fungi

III.1.3.3.1. Uv Tansilluminator temperature fluctuation

The temperature under the trans-illuminator increased with exposure time. For exposure time ranged between 0 min – 45 min temperature was 25 – 43.12 ◦C (25.00 ± 0.41, 29.90 ± 1.26, 38.25 ± 0.77 and 43.12 ± 0.37◦C respectively after 0, 15, 30 and 45 min exposure to UV light in trans-illuminator).

III.1.3.3.2. Effect of UVB light on conidia germination

Results showed positive correlation between the number of colonies formed 4 DPI and 10 DPI ($t = 435.50$; $df = 30$; $P < 0.001$; $R = 0.99$). The number of colonies increases when the time of incubation increases. It also revealed that when exposure time under UVB light increases the conidia survival decreases (table XI).

Data of 4 DPI showed that exposure time and interaction exposure time - isolate affected significantly the number of colonies formed units ($F = 224$; $df = 3$; $P < 0.001$ and $F = 42.3$; $df = 3$; $P < 0.001$ respectively). However, the number of colonies formed units were not significantly different between isolates ($t = 0.79$; $df = 30$; $P = 0.44$); when expose for 0 -45 min, colony formed unit ranked 62.50 ± 12.50 -3050 ± 222.70 cfu and $0 - 2025 \pm 22.70$ cfu for BIITAC6.2.2 and MIITAC11.3.4 respectively (Table XI).

At 10 DPI, same trend was observed, colonies were significantly different between exposure time ($F = 193.14$; $df = 3$; $P < 0.001$) and interaction isolate-exposure time ($F = 39.40$; $df = 3$; $P < 0.001$) and no significant difference occurred between isolates ($t = 0.8$; $df = 30$; $P = 0.43$). Conidia of BIITAC6.2.2 tolerated exposure under UVB light for 45min while at no colony were formed at this exposure time even when incubation time was prolonged of 6 days for MIITAC11.3.4.

Table XI. UVB light action on conidia viability at different exposure time, four and ten-days post-incubation

Exposure time	BIITAC6.2.2		MIITAC11.3.4	
	4dpi	10dpi	4dpi	10dpi
0 min	$(3.05 \pm 0.22) \times 10^3$ a	$(3.05 \pm 0.22) \times 10^3$ a	$(2.02 \pm 0.60) \times 10^3$ a	$(2.02 \pm 0.60) \times 10^3$a
15 min	$(0.62 \pm 0.12) \times 10^2$ b	$(0.68 \pm 0.11) \times 10^2$ b	$(0.50 \pm 0.1) \times 10^2$ b	$(0.62 \pm 0.16) \times 10^2$ b
30 min	$(0.92 \pm 0.15) \times 10^2$ b	$(1.25 \pm 0.20) \times 10^2$ b	$(0.25 \pm 00) \times 10^2$ b	$(0.31 \pm 0.06) \times 10^2$ c
45 min	$(1.37 \pm 0.52) \times 10^2$ b	$(1.43 \pm 0.50) \times 10^2$ b	0 c	0 d
	$F = 33.97$; $df = 3$; $P < 0.001$	$F = 37.73$; $df = 3$; $P < 0.001$	$F = 33.60$; $df = 3$; $P < 0.001$	$F = 773.3$; $df = 3$; $P < 0.001$

dpi = days post-inoculations;
Means followed by the same letter in the same column are not significantly different with Tukeys HSD test, $\alpha = 5\%$.

III.1.3.3.3. Effect of UVB light on entomopathogenic fungi mycelia growth

In this study, exposure time and interaction isolate-exposure time under UVB light had a high significant effect on colony size ($F = 19.40$; $df = 3$; $P < 0.001$ and $F = 17.84$; $df = 3$; $P < 0.001$ respectively). The mycelia growth of BIITAC6.2.2 and MIITAC11.3.4 after 0 – 45 min exposure under UVB light were respectively 0.95 ± 0.05 – 2.36 ± 0.13mm/day and 1.47 ± 0.07 – 2.16 ± 0.10mm/day (fig. 26). the optimum growth was achieved when colonies were exposed for 30 min under UVB light for BIITAC6.2.2 while exposure under UVB light did not affect mycelia growth of MIITAC11.3.4.

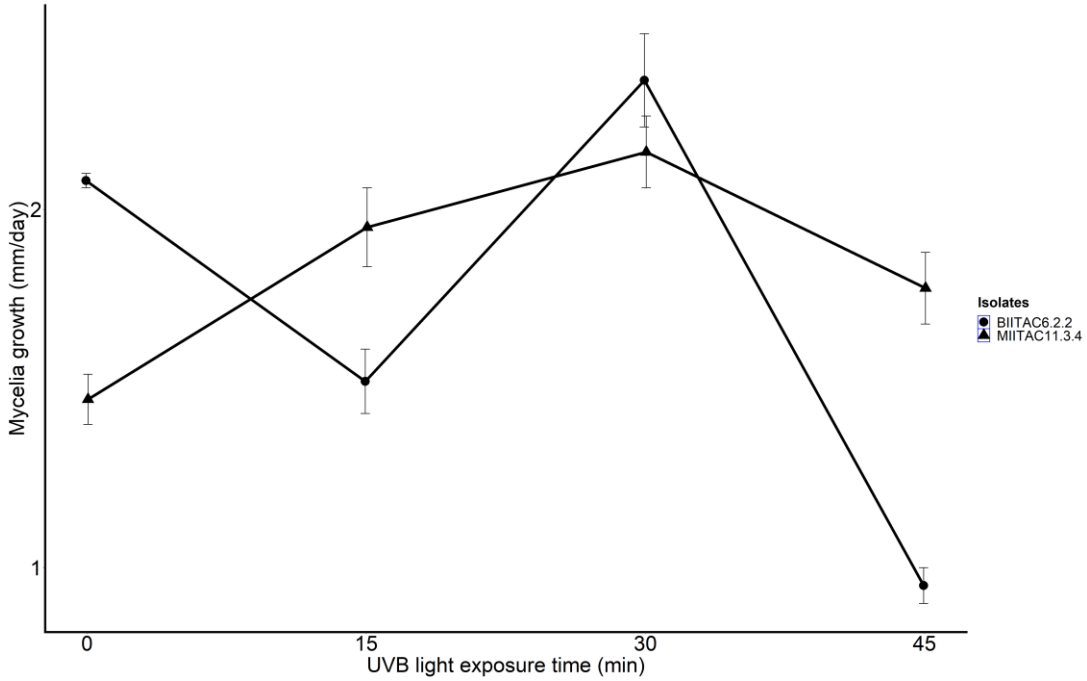

Fig. 26. Mycelial growth of fungal isolates at different exposure time under UVB light on PDA media after 15 days of incubation

III.1.3.3.4 Effect of UVB light on EPF conidia yield

When conidia were produced on PDA media under ultra violet light B (UVB) at different exposure time, the conidia production were significantly affected by exposure time ($F = 72.43$; $df = 3$; $P < 0.001$) and by interaction exposure time – isolate ($F = 61.84$; $df = 3$; $P < 0.001$). No significant difference was found between isolates ($t = 1.60$; $df = 30$; $P = 0.12$). The conidia reproduction of Beauveria BIITAC6.2.2 was ranked $2.64 \pm 0.08 \times 10^7$ conidia/mL

– 5.58 ± 0.2 x 10^8 conidia/mL) with high amount of conidia produced at 0 min exposure followed by 30 minutes' exposure under UVB light (F = 458.5; df=3; P < 0.001). For Metarhizium MIITAC11.3.4, conidia production was similar at all exposure times under UVB light (F = 1.006; df=3; P = 0.42; fig. 27).

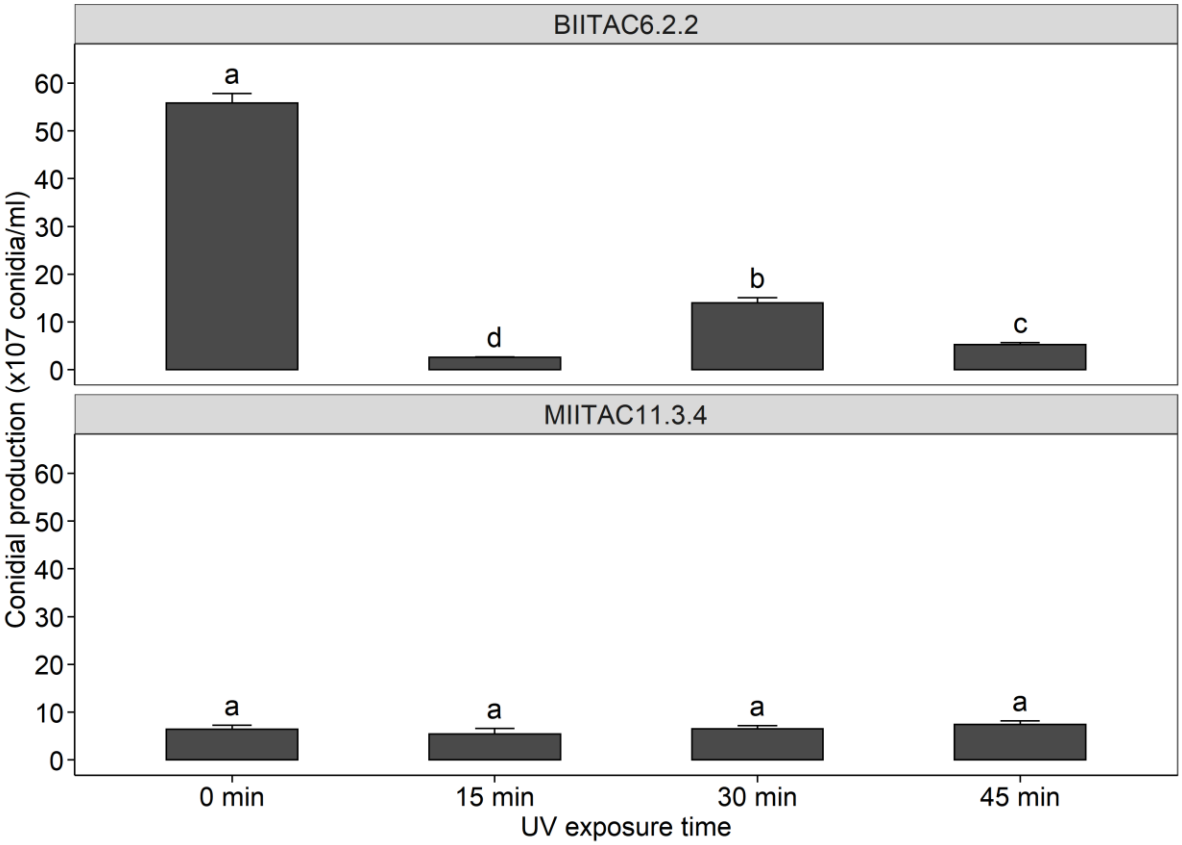

Fig. 27. Conidia yield of fungal isolates at different exposure time under UVB light on PDA media after 15 days post-incubation.

III.1.3.4 Effect of relative humidity on fungal isolates

III.1.3.4.1 Effect of relative humidity on EPF conidia germination

The number of colony formed unit were 00-3050 ± 200 cfu and 250 ± 17 – 3110 ± 200 for BIITAC6.2.2 and MIITAC11.3.4 respectively (table XVI). However, there was no significant difference between isolates (t = -0.33; df = 30; P = 0.73), while relative humidity (F = 3013; df = 3; P < 0.0001) and interaction isolate-relative humidity (F = 4645; df = 3; P < 0.0001) affected conidia formation. The highest number of colony formed unit were counted at relative humidity 75 % (3050 ± 200 cfu) and 99 % (2660 ± 20 cfu) for BIITAC6.2.2 and 30

% (3110 ± 200 cfu) MIITAC11.3.4 with no significant difference between 75 and 99% relative humidity (Table XII).

Table XII. Conidia viability of fungi strains under different relative humidities

RH (%)	BIITAC6.2.2	MIITAC11.3.4
30	00 ± 00 c	(3.11 ± 0.20) x10^3 a
50	(1.10 ± 0.01) x10^3 b	(2.5 ± 0.17) x10^2 c
75	(3.05 ± 0.20) x10^3 a	(2.02 ± 0.06) x10^3 b
99	(2.66 ± 0.02) x10^3 a	(1.98 ± 0.02) x10^3 b
	F= 10347, df= 3, P < 0.001	F= 458, df= 3, P < 0.001

RH= relative humidity;
Means followed by the same letter in the same column are not significantly different with the Tukeys HSD test, α = 5%.

III.1.3.4.2 Effect of relative humidity on EPF mycelial growth

All tested relative humidity permitted mycelia growth of both MIITAC11.3.4 and BIITAC6.2.2. However, mycelial growth varied significantly between isolates (t = 3.66; df = 446; P < 0.001), relative humidity (F = 23.86, df = 3; P <0.001) and interaction Isolate-relative humidity (F = 23.82; df = 3; P < 0.001). The colony diameter varied from 1.52 to 2.96 mm/day and from 1.57 to 2.26 mm/day for BIITAC6.2.2 and MIITAC11.3.4 respectively (fig. 28). The optimum growth for BIITAC6.2.2 were respectively at relative humidity 30 % (2.96 ± 0.13 mm/day) followed by 50 and 75 % relative humidities which showed similar trend. Mycelial growth of MIITAC11.3.4 was significantly higher at 50 % with no difference between 30 and 99 % relative humidities.

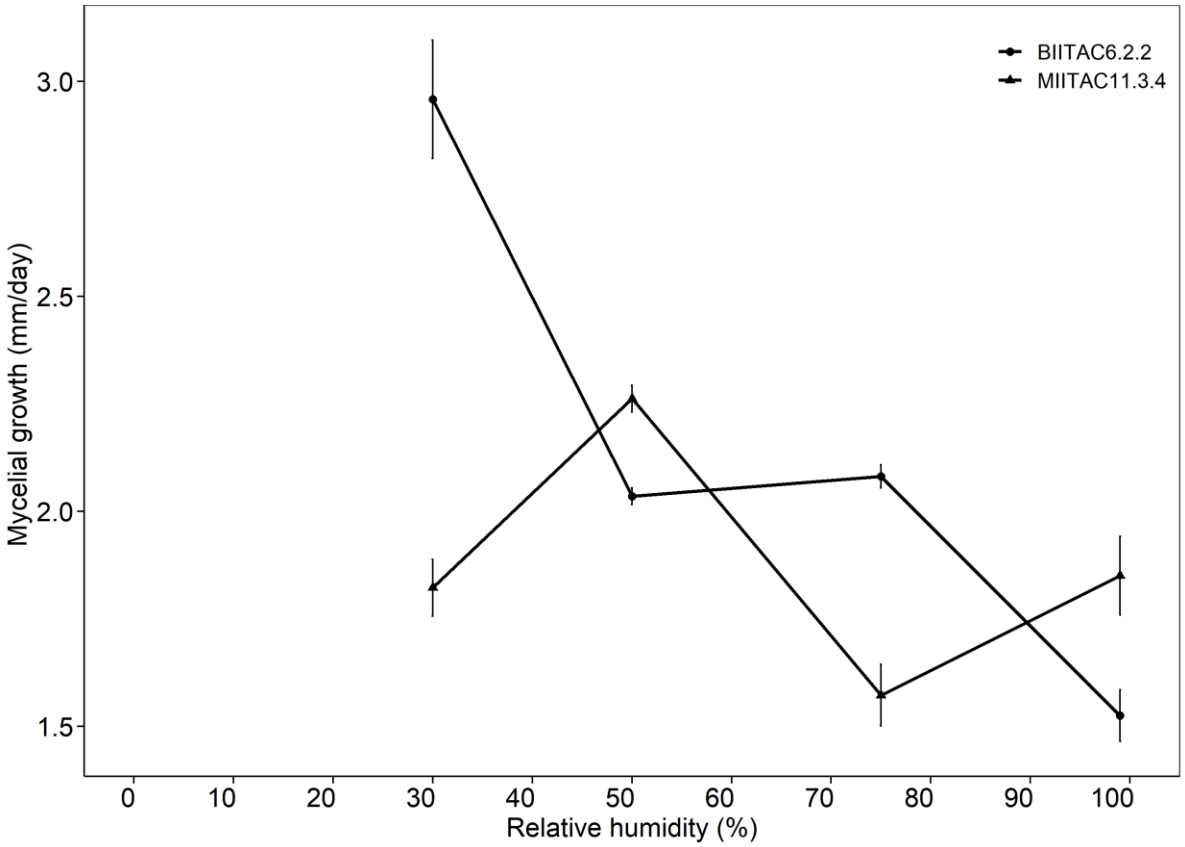

Fig. 28. Mycelia growth of fungal isolates at different relative humidities of PDA media 15 days post-incubation.

III.1.3.4.3. Effect of relative humidity on EPF conidia yield

Results revealed that there was significant difference between isolates ($t = 3.60$; $df = 30$; $P = 0.001$), between relative humidity ($F = 42.36$; $df = 3$; $P < 0.001$) and in interaction relative humidity – isolate ($F = 12.56$; $df = 3$; $P < 0.001$). Beauveria BIITAC6.2.2 showed high conidia production at RH 75 % (4.13×10^8 conidia/mL) followed by RH 50 and 99 % which had same trend ($F= 40.24$; $df=3$; $P < 0.001$) (fig. 29). For Metarhizium MIITAC11.3.4, high conidia reproduction was obtained at RH 50 and 75 % , $1.02 \pm 0.13 \times 10^8$ and $4.92 \pm 0.5 \times 10^7$ conidia/mL respectively ($F= 17.28$; $df=3$; $P < 0.001$).

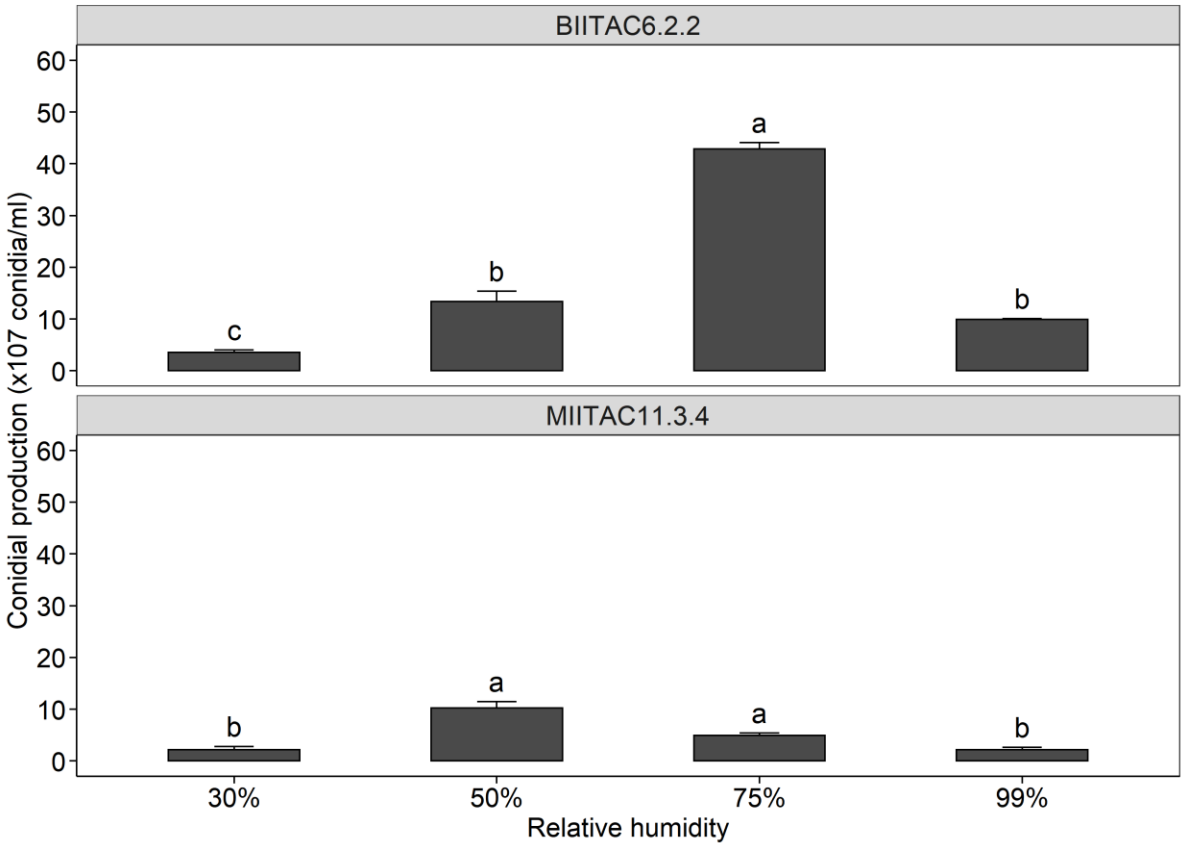

Fig. 29. Conidia yield of fungal isolates under different relative humidities after 15 days of incubation.

III.1.4. Screening of ingredients mycoinsecticide formulation

III.1.4.1. Quality of active ingredient

The uniformity of both entomopathogenic fungi spore powder revealed their purity (Table XIII). conidia yield in dry powder showed considerable variation between biotypes ($P < 0.0001$). The *B. bassiana* BIITAC6.2.2 showed a high amount of conidia per gram of dry conidia powder compare to *M. anisopliae* MIITAC11.3.4. Besides, the conidia viability of Beauveria isolate was also significantly high (92 %) compare to Metarhizium isolate ($P < 0.001$). When spread on the PDA plate, only Metarhizium isolate was contaminated with filamentous fungus namely Rhizopus sp. However, 30 days post-exposure to conidia suspension tired 3.2 x 10^8 conidia/ml, mortality and mycosis rate did not differ significantly between fungi isolates (Mortality: $t = 1.006$; $df = 10$; $P = 0.34$; Mycosis: $t = 1$; $df = 10$; $P =$

0.34). The mortality rate of BIITAC6.2.2 and MIITAC11.3.4 were 63.33 and 50 % respectively while mycosis rates were 100 and 83.33 %.

Table XIII. Characteristic of mass-produced conidia dry powders

	Active ingredients	
Quality control parameters	BIITAC6.2.2	MIITAC11.3.4
Appearance	Uniform cream powder	Uniform green powder
Viability (%)	92.00 ± 0.70 a	35.25 ± 3.50 b
contaminants	absent	present (*Rhizopus* sp)
Number of conidia/g	$2.85 \times 10^{11} \pm 9.43 \times 10^{10}$ a	$2.06 \times 10^{10} \pm 2.94 \times 10^{8}$ b
Mortality rate	63.33 ± 6.14 a	50.00 ± 12.4 a
Mycosis rate	100 ± 00 a	83.33 ± 16.67 a

Means followed by the same letter in the same column are not significantly different with the Tukeys HSD test, $\alpha = 5\%$.

III.1.4.2. Characteristic of inactive ingredients for formulation development

III.1.4.2.1 **Compatibility** of inactive ingredients with conidia

Conidia viability varied significantly between strains ($F = 1479.43$; $df = 1$; $P < 0.001$) and storage time ($F = 626.30$; $df = 2$; $P < 0.001$).

For Beauveria BIITAC6.2.2, conidia germination reduced with time except in pre-formulation F2 and F4 where viability increased instead. Table XIV showed that low reduction of germination was obtained principally for F5 and F12, 0.40 % and 6.74 % respectively after 8 weeks of incubation at 4 ◦C. these formulations were followed by F8, F9, and F10. The viability of unformulated conidia was reduced by 14.40 % at 8 weeks post-incubation. Conidia viability was significantly affected by formulations ($P < 0.001$). The pre-formulations F4, F5, F8, F9, F10, F12 showed significant high conidia viability at 8 weeks post-incubation, which was not significantly different from F2. The high values of LT80 was obtained for F8 (58.86 weeks), F9 (23.53 weeks), F12 (23.47 weeks), F5 (22.73 weeks), F10

(21.01 weeks) F2 (15.79 weeks), and F4 (12.99 weeks) compare to unformulated conidia (9.28 weeks).

For MIITAC11.3.4, Conidia viability was reduced for both formulated and unformulated conidia. Pre-formulation F5 exhibited the highest conidia viability (17.25 %) and the lowest reduction percentage (38.53 %) after 8 weeks of storage at 4 °C (Table XIV). The conidia viability of pre-formulations F12, F9, F10, F1, and F4 seems to be not harmful to conidia, with a germination rate of 15.75, 15, 11, 11 and 9.25 % compare to unformulated conidia (9 %). Their percentage reduction was ranged 54.78 - 65.99 % compare to conidia viability of unformulated conidia (74.39 %) after 8 weeks of storage. Pre-formulations F11, F2, and F3 inhibited conidia germination at 91.50, 95.72, and 100 % respectively.

III.1.4.2.3. Water holding capacity or porosity

There were significant differences between carriers ($F = 1435$; $df = 11$; $P < 0.001$). The lowest value of WHC was 0.76, 0.84, and 0.94 with kaolin clay, cassava starch, and wood ash respectively (fig. 30). Most nutritive carriers showed high WHC above1.3 except rice flour (1.12) banana flour (1.19) and maize flour, indicating their fast humectation and long conservation of humidity.

III.1.4.2.4 Physical uniformity

III.1.4.2.4.1 Granulometry

The granulometric distribution (fig. 31) showed that all particles passed in the 2000µm sieve while 0 - 18.61 % of 5 g sample was retained in the 500 µm. Based on the carrier's retention in the 150-µm sieve, ingredients were group into four categories: i) carriers of moderate coarse particles of 87.28 - 99.91 % particles retained in the 150-µm sieve (C2); ii) carriers of moderate fine particles with 67.74 - 81.30 % particles retained (C8, C1, C11, C9); iii) Carriers of fine particles with 49.60 - 65.8 % particles retained (C5, C7, C3) and iv) carriers of very fine particles with 67.74 - 81.30 % particles retained (C4, C10, C12, C6). Among carriers of very fine particles, the ingredients C4, C10, C12, C6, and C5 retained respectively 22.01, 28.12, 36.69, and 47.38 % particles in the 100 µm sieve.

Table XIV. Shelflife of preformualtions at 4 °C

Pre-formulations	BIITAC 6.2.2				MIITAC 11.3.4			
	Conidia viability (%)			Viability reduction (%)	Conidia viability (%)			Viability reduction (%)
	0WPI	4WPI	8WPI		0WPI	4WPI	8WPI w	
F0	92.00 ± 0.71 cde	89.50 ± 1.55 ab	78.75 ± 0.85 bc	14.40 ± 1.21 cde	35.25 ± 3.50 bc	18.50 ± 1.93 a	9.00 ± 1.47 abc	74.40 ± 4.20 abcde
F1	93.00 ± 0.70 bcde	87.50 ± 1.65 ab	76.5 ± 1.32 cd	17.70 ± 1.90 bcd	35.00 ± 1.91 bc	11.50 ± 2.33 a	11.00 ± 2.12 abc	67.53 ± 8.20 bcde
F2	76.50.00 ± 3.75 f	82.50 ± 3.09 ab	84.50 ± 2.39 ab	-10.80 ± 2.26 g	34.50 ± 1.85 bc	9.25 ± 4.73 ab	1.50 ± 0.50 de	95.72 ± 1.30 ab
F3	93.50 ± 0.95 bcd	66.75 ± 1.12 c	68.25 ± 1.93 de	27.03 ± 1.51 b	8.00 ± 1.08 d	00 ± 00 c	00 ± 00 e	100 ± 00 a
F4	70.25 ± 2.21 f	87.00 ± 2.90 ab	87.00 ± 1.08 a	-24.30 ± 4.94 h	27.00 ± 1.80 c	10.75 ± 1.65 ab	9.25 ± 1.65 abc	66.00 ± 5.04 cdef
F5	88.250 ± 1.79 de	88.00 ± 1.08 ab	87.75± 0.85 a	0.40 ± 2.97 fg	28.75 ± 1.65 c	10.75 ± 0.75 ab	17.25 ± 2.50 a	38.51 ± 11.62 f
F6	96.75 ± 1.11 ab	79.00 ± 1.50 bc	74.50 ± 1.32 cde	22.96 ± 1.75 bc	26.50 ± 1.32 c	12.00 ± 1.08 a	6.25 ± 1.31 bcd	76.90 ± 3.74 abcd
F7	87.00 ± 0.91 e	69.25 ± 1.40 c	66.25± 1.11 e	23.80 ± 1.94 bc	30.25 ± 1.65 bc	9.00 ± 1.80 ab	8.25 ± 1.25 abc	72.95 ± 3.60 abcde
F8	97.50 ± 0.50 ab	88.75 ± 1.25 ab	88.75± 0.85 a	9.00 ± 0.65 def	40.50 ± 2.02 b	13.25 ± 2.62 a	6.75 ± 2.40 cd	54.80 ± 3.75 abcd
F9	98.50 ± 0.29 a	91.25 ± 2.90 a	86.25 ± 0.75 a	12.42 ± 1.01 cde	33.00 ± 0.81 bc	15.25 ± 3.77 a	15.00 ± 1.60 ab	67.60 ± 8.20 def
F10	96.25 ± 0.75 abc	89.75 ± 1.11 ab	88.75± 1.75 a	7.76 ± 2.10 def	33.75 ± 3.04 bc	12.25 ± 1.20 a	11.00 ± 1.60 abc	67.00 ± 4.61 bcdef
F11	87.50 ± 0.64 de	22.25 ± 4.57 d	78.75± 0.85 bc	63.70 ± 2.50 a	56.00 ± 00 a	3.00 ± 1.15 bc	4.75 ± 0.50 cd	91.52 ± 0.85 abc
F12	93.75 ± 0.75 bcd	92.00 ± 1.30 a	88.00 ± 1.73 a	6.12 ± 1.84 ef	30.00 ± 2.70 c	13.25 ± 0.85 a	15.75 ± 2.66 ab	46.83 ± 9.21 f
	F = 35.83; df = 12; P< 0.001	F = 49.77; df = 12; P < 0.001	F = 80.25; df = 12; P < 0.001	F = 83.07; df = 12; P < 0.001	F = 31.05; df = 12; P < 0.001	F = 10.34; df = 12; P < 0.001	F = 13.20; df = 12; P < 0.001	F = 9.96; df = 12; P < 0.001

WPI= week post-incubation.

Means followed by same letter in the same column are not significantly different with the Tukeys HSD test, $\alpha = 0.05$; F0= unformulated conidia and pre-formulations F1= rice flour; F2= Gari; F3= carrot powder; F4= Wood ash; F5= kaolin clay; F6= Baoba powder; F7= Mucuna leaf powder; F8= charcoal powder; F9= maize flour; F10= cassava starch; F11= milk powder; F12= banana flour.

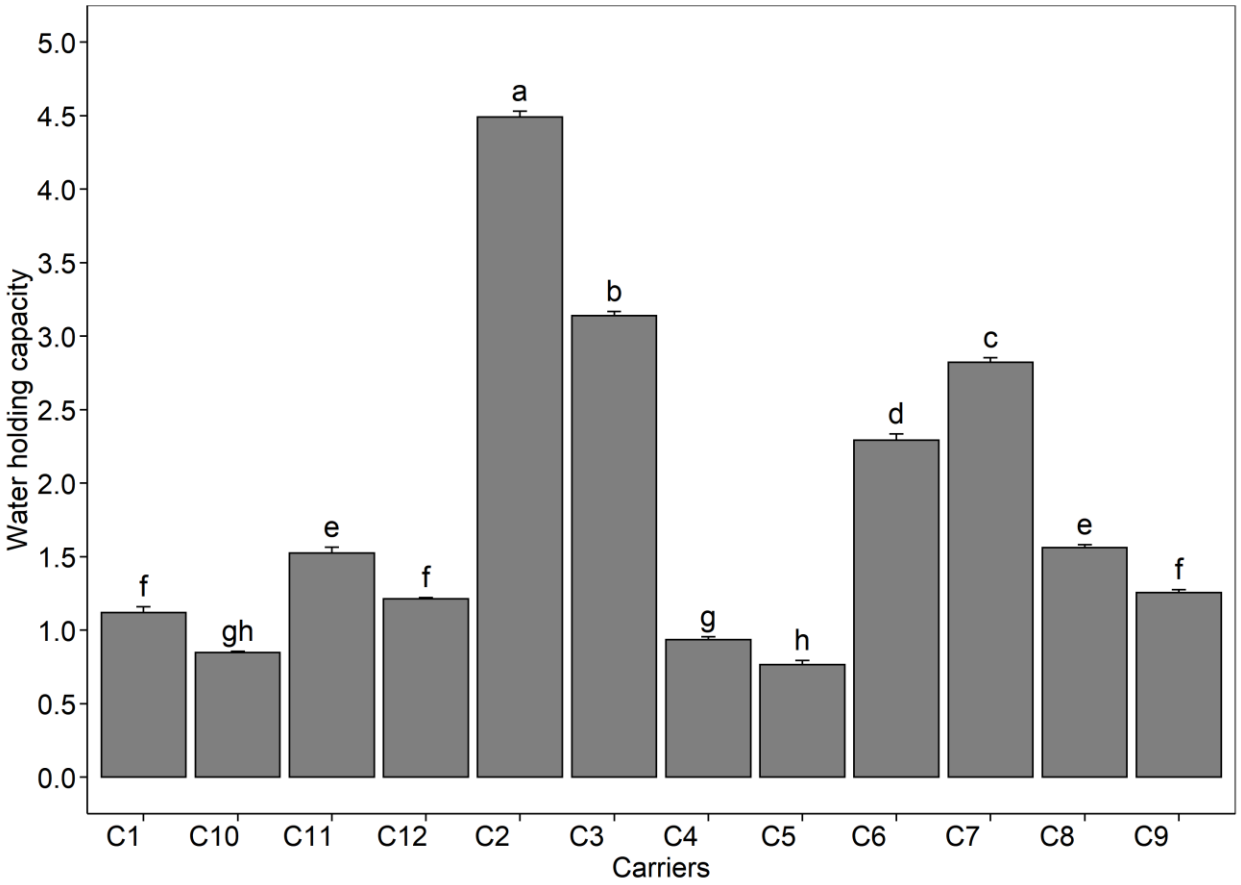

Fig. 28. Water holding capacity of each carrier.

C1= rice flour; C2= Gari; C3= carrot powder; C4= Wood ash; C5= kaolin clay; C6= Baoba powder; C7= Mucuna leaf powder; C8= charcoal powder; C9= maize flour; C10= cassava starch; C11= milk powder; C12= banana flour. Means followed by same letter are not significantly different with the Tukeys HSD test, $\alpha = 0.05$;

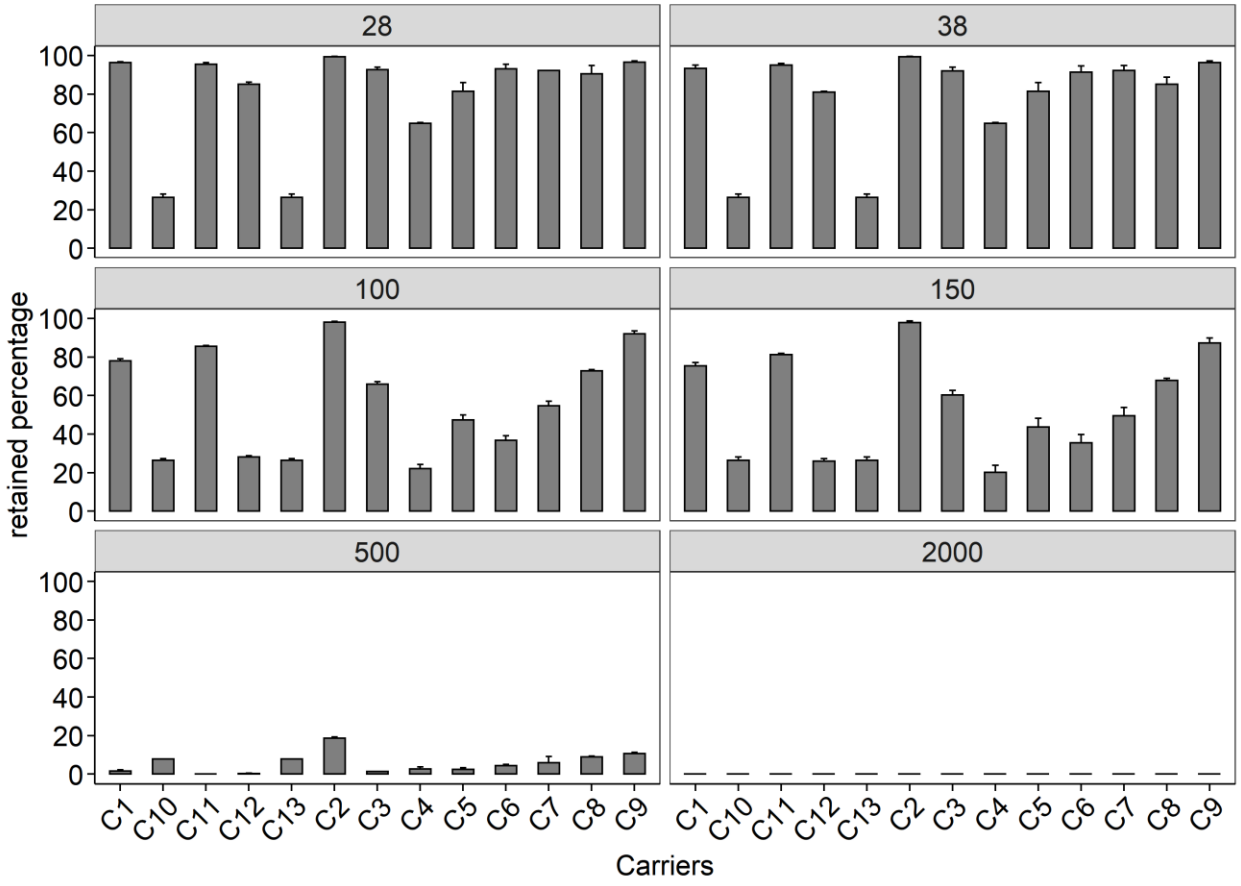

Fig. 29. Granulometric distributions of carriers by sieve mesh (µm)

C1= rice flour; C2= Gari; C3= carrot powder; C4= Wood ash; C5= kaolin clay; C6= Baoba powder; C7= Mucuna leaf powder; C8= charcoal powder; C9= maize flour; C10= cassava starch; C11= milk powder; C12= banana flour.

III.1.4.2.4.2. Flow properties

The CI and HR indicate the compression capacity of powders and their flowability. From the results of the measurement of flow properties, it appeared that followability depends on carriers. The values of Hausner ratio ($F = 64.37$; $df = 11$; $P < 0.001$) and Carr's index ($F = 58.28$; $df = 11$; $P < 0.001$) were significantly different between carriers, with rank between 1.05 - 1.96 and 4.87 - 48.89 respectively. The lowest value of HR and CI were for C1 and C2 indicating excellent flow. The carriers C4 and C8 showed fair flowability with HR and CI values of 1.23 and 18.46 %, 1.25, and 19.94 % respectively. Thus, C5, C10, C9, and C12 were characterized as poor flow powders (Table XV).

Table XV. Flow ability properties of formulation ingredients

Ingredients	HR	CI	Flow character	pH (± SE)	RH (% ± SE)
Rice flour	1.09	8.17	excellent	6.19± 0.003e	12.1 ± 0.153c
Gari	1.05	4.87	excellent	4.35 ± 0.02b	16.33 ± 0.18e
Carrot powder	1.58	36.56	very poor	4.97 ± 0.00c	*
Wood ashes	1.25	19.94	fair	11.04 ± 0.01i	14.27 ± 0.12d
Kaolin clay	1.43	30.16	poor	4.48 ± 0.00b	14.77 ± 0.18d
Baobab powder	1.62	38.26	very very poor	3.16 ± 0.007a	*
Mucuna powder	1.58	36.87	very poor	5.05 ± 0.00c	*
Charcoal	1.23	18.46	fair	9.71 ± 0.00h	27.33 ± 0.58f
Maize flour	1.61	37.78	very very poor	6.25 ± 0.003e	11.6 ± 0.00c
Cassava starch	1.96	48.89	very very poor	6.48 ± 00f	7.77 ± 0.14b
Milk powder	1.34	25.27	fair	6.72 ± 0.01g	*
Banana flour	1.62	38.33	very very poor	5.86 ± 0.003d	6.43 ± 0.18a

HR=Hausner ratio; CI= Carr's index; RH= relative humidity, *= no data; Means followed by same letter in the same column are not significantly different with the Tukeys HSD test, $\alpha = 0.05$

III.1.4.2.4.3. Neutral pH and moisture content

The pH values of carriers were ranged from 3.16 to 11.14. Significant differences were observed between carriers ($F = 4793$; $df = 11$; $P < 0.001$) with higher values for C8 (Charcoal) and C4 (wood ash), indicating basicity. Moreover, lower values of pH were obtained with C6 (baobab powder) followed by C2 (Gari flour), C5 (kaolin clay), C7 (Mucuna leaves), and C12 (Banana flour), indicating acidity. No carrier showed neutral PH but C11 followed by C10, C9 and C1 were nearby neutral pH (Table XV).

The one-way ANOVA analysis showed that moisture content ranged from 6.43 to 27.33 %, differ significantly between carriers ($F = 669.8$; $df = 7$; $P < 0.001$). Banana flour (C12) and

cassava (C10) were the drier carriers with 6.43 and 7.76 % humidity respectively (Table XV). Charcoal was the wet carrier (27.33 % relative humidity).

III.1.4.2.4.4. Economic value and availability

Based on the table below, all the ingredients used in our studies were found in target markets except Mucuna and wood ash. The available ingredients were not all in the usable form (powder or flour) except C2, C9, C10, and C11 (Table XVI).

The cost of carriers varied significantly ($F = 567.9$; $df = 10$; $P < 0.001$). The price was between 233.33 and 6166.67 FCFA. The cheaper ingredients were C5 and C8 which cost 233.33 and 266.67 FCFA respectively. The cost of C4, C12, C9, and C10 was not significantly different (Table XVI).

III.1.4.2.4.5. Selection of UV protectant for dust-based formulations

There was not significant variability in the tolerance to UV-B radiation between *B. bassiana* and *M. anisopliae* Isolates ($F = 0.42$; $df = 1$; $P = 0.51$). However, germination rate after exposure and the percentage of original activity remaining (OAR) were significant difference between formulations ($F = 17.55$; $df = 12$; $P < 0.001$ and $F = 42.36$; $df = 12$; $P < 0.001$ respectively). As result of OAR on Beauveria conidia, there was significant difference between pre-formulation ($F = 224.42$; $df = 12$; $P < 0.001$). The percentage of original activity remain also varied significantly between formulated and unformulated conidia ($F = 25.17$; $df = 12$; $P < 0.001$).

The percent original activity of the twelve formulations was ranged from 0 % (F2 and F7) to above 75 % (F8, F0, F12, F5, and F10) for both Beauveria and Metarhizium strains (fig. 32). Five (F2, F7, F11, F3, and F6) and four ingredients provided respectively less than 50 % protection, and between 50 – 77 % protection for conidia of both strains. The ingredients which provided more than 75 % protection to both strains were selected for the secondary screen with sunlight.

Table XVI. Availability and price of inactive ingredients according to targets markets

Ingredients	Price of 1Kg (FCFA)	Availability
Rice flour	516.67 ± 44.09ab	(++)
Gari	550 ± 50.00ab	(+++)
Carrot powder	3166.67 ± 166.67d	(+)
Wood ashes	466.67 ± 33.33ab	(+)
Kaolin clay	233.33 ± 133.33a	(++)
Baobab powder	2200.00 ± 152.75c	(++)
Mucuna powder	*	(-)
Charcoal	266.67 ± 33.33a	(+++)
Maize flour	566.67 ± 16.67ab	(+++)
Cassava starch	700 ± 00ab	(+++)
Milk powder	6166.67 ± 16.67e	(+++)
Banana flour	500.00 ± 00b	(+)

*not determine; Not (-) not available; (+++) high available in the form needed; (++) high available but not in the form needed; (+) less available but not in the form needed.
Values followed by the same letter in the same column are not significantly different (*P <0.05)*.

Results post exposure to sunlight revealed that there was significant difference on percentage of original activity and all ingredients show more than 90 % protection except F12 (6.69 % OAR) and F0 (57.53 % protection) (fig. 33).

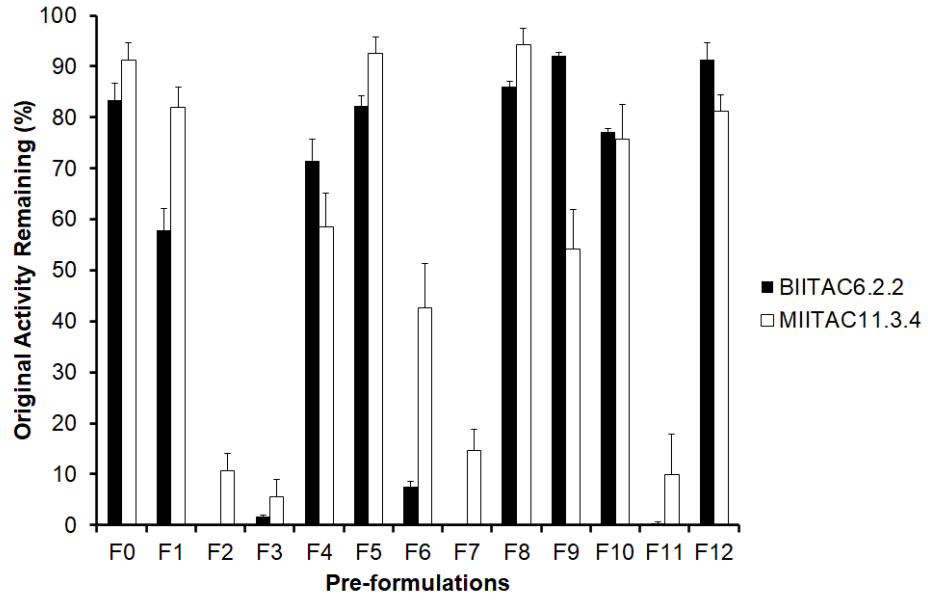

Fig. 30. Original activity remaining after one-hour of pre-formulation exposure under UVB light.
F0= Unformulated conidia; F1= rice flour; F2= Gari; F3= carrot powder; F4= Wood ash; F5= kaolin clay; F6= Baoba powder; F7= Mucuna leaf powder; F8= charcoal powder; F9= maize flour; F10= cassava starch; F11= milk powder; F12= banana flour.

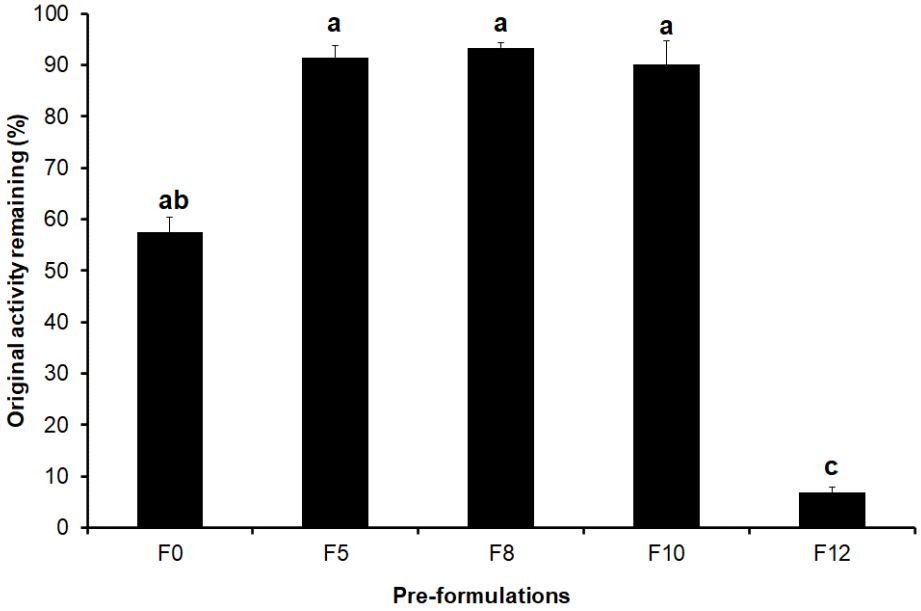

Fig. 31. Original activity remaining after one-hour of pre-formulation exposure under sunlight.
F0= Unformulated conidia; F1= rice flour; F2= Gari; F3= carrot powder; F4= Wood ash; F5= kaolin clay; F6= Baoba powder; F7= Mucuna leaf powder; F8= charcoal powder; F9= maize flour; F10= cassava starch; F11= milk powder; F12= banana flour; Means followed by same letter are not significantly different with the Tukeys HSD test, $\alpha = 0.05$;

III.1.5. Mycoinsecticide characteristics

III.1.5.1. Physico-chemical characteristic of formulations

The water holding capacity (WC) of co-formulants was between 0.68-1.45. The lesser values of absorption capacity were obtained for CO-F1 (0.68) and CO-F4 (0.83). The results of physical uniformity revealed that CO-F3 had good flowability properties (CI and HR respectively 15.17 and 1.18). However, adjuvants' pH was between 6.26 and 8.49. The lowest moisture content was approximately 9.42 % and the highest 13.86 % in CO-F3 and CO-F1 respectively (table XVII). Formulations had approximatively the same price between 5673 and 6200 FCFA.

Table XVII. Physico-chemical properties of co-formulation

Parameters	Co-Formulations			
	Co-F1	Co-F2	Co-F3	Co-F4
WC	0.68 ± 0.02	1.36 ± 0.04	1.45± 0.1	0.83 ± 0.01
Uniformity CI	33.83 ± 0.98	46.98 ± 1.71	15.17 ± 1.81	29.40 ± 0.30
Uniformity HR	1.51 ± 0.02	1.89 ± 0.06	1.18 ± 0.02	1.42 ± 0.01
pH	8.01 ± 0.02	6.26 ± 0.06	7.53 ± 0.02	8.49 ± 0.02
RH	13.86 ± 0.07	9.60 ± 0.14	9.42 ± 0.10	11.48 ± 0.04
Formulation cost (FCFA)	5673	6200	5862	6010

RH= relative humidity ; CO-F1= kaolin clay based co-formulation; CO-F2= maize flour based co-formulation ; F3= banana flour based co-formulation; CO-F4 = cassava starch based co-formulation.

III.1.5.2. Shelf-life of formulations

Results showed that conidia viability was significantly different between temperature ($F = 202.22$; $df = 3$; $P < 0.001$). Conidia viability was not significantly affected by temperatures 4°C, -20 °C and -50 °C. At these temperatures, conidia germination of formulated and unformulated conidia was maintained above 90 % after 72 weeks' storage whereas, spore's germination decreased fast at 25 °C, losing 100 % viability after 40 weeks for formulated and unformulated conidia (fig. 34). However, incubation time affected drastically conidia viability at 25 °C only. Generally, there were no significant difference between formulated and unformulated conidia ($F = 0.024$; $df = 4$; $P = 0.99$).

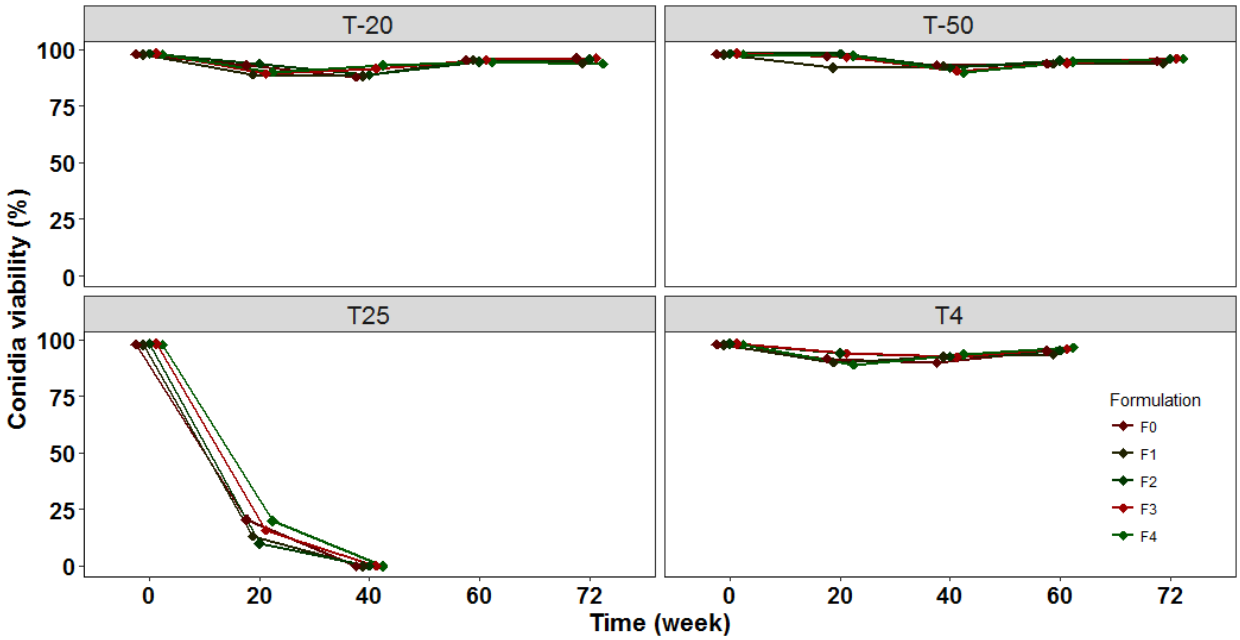

Fig. 32. Shelf-life of formulations under different storage conditions (4, 25, -20, and - 50 ◦C).
T-20= temperature -20 °C; T-50= temperature -50 °C; T25= temperature 25 °C; T4= temperature 4°C; F0= unformulated conidia; F1 = kaolin clay based formulation; F2 = maize flour based formulation ; F3 = banana flour based formulation;F4 = cassava starch based formulation.

III.1.5.3. Bio-efficacy of formulation in laboratory conditions

Fourteen days after application of treatment on adult weevil, data of mortality and mycosis showed that there were significant differences between treatments ($F = 23.77$; $df = 9$; $P < 0.001$ and $F = 35.02$; $df = 9$; $P < 0.001$). Co-formulants caused mortality up to 25 % while control was responsible for only 15 % mortality (fig. 35). The mortality of co-formulant and control was not significantly different. None of the dead insects from co-formulant and control treatments showed fungi out growth.

All formulations caused up to 100 % mortality after 14 days whereas non-formulated conidia caused 93.33%. The highest mortality (100 %) was obtained with formulation F2 followed by F1 (96.67 %) and F4 (96.67 %). High infection rates were obtained with formulation F4 (93 %)and F1 (90 %).

The values of LT50 and LT90 were ranged between 4.80 - 7.66 days and 8.57 - 13.51 days. Formulation F4 caused rapidly 50 and 90 % mortality of adult weevils. The average conidia yield showed that all formulations initiate on insect sporulation above 10^7 conidia/ml. However, insects treated with F0 (unformulated conidia) followed by F1 and F2 produced a high quantity of conidia (Table XVIII).

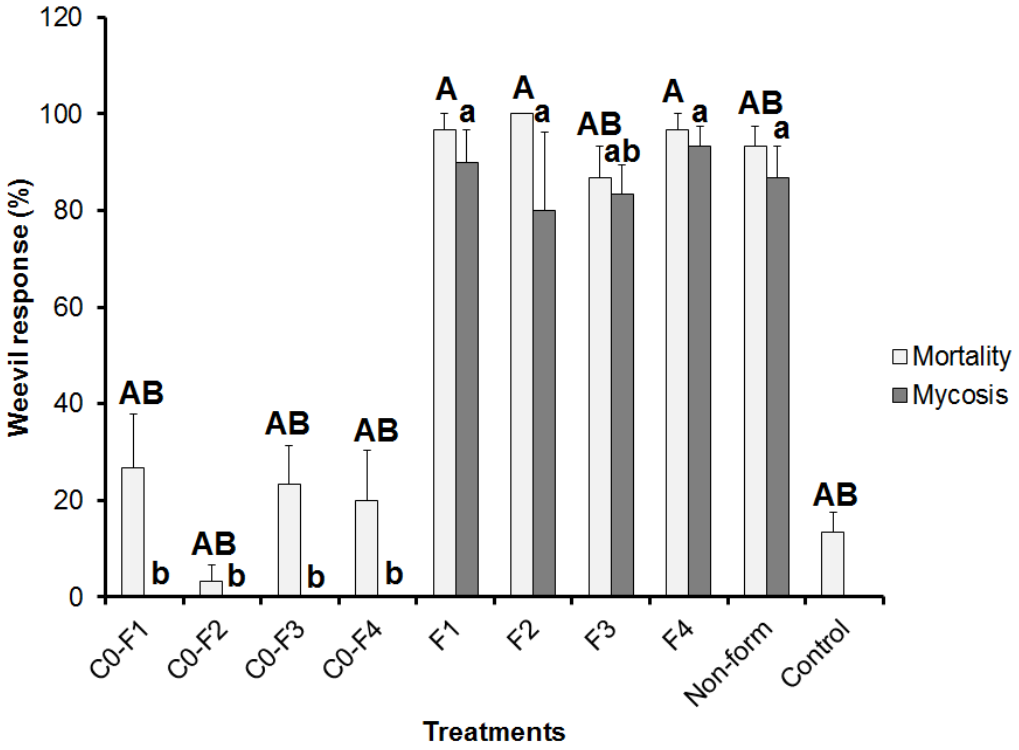

Fig. 33. Response of biopesticide treatments 14 days' post-exposure. Different uppercase letter meaning a significant difference in mortality between treatments; different lowercase letter meaning a significant difference in mycosis between treatments.

Table XVIII. Lethal time and conidia yield from insects treated with the formulation in the laboratory

Treatment	LT50 (days)	LT90 (days)	Conidia yield/insect
F1	6.30 (5.45 - 7.12)	10.49 (9.09 - 13.06)	$1.08x10^{8} \pm 1.45x10^{7}$b
F2	6.05 (5.27 - 6.82)	9.96 (8.66 - 12.30)	$1.06x10^{8} \pm 2.39x10^{7}$c
F3	7.66 (6.60 - 8.77)	13.51 (11.36 - 18.27)	$4.37x10^{7} \pm 8.498x10^{6}$e
F4	4.80 (4.30 - 5.29)	8.57 (7.69 - 9.86)	$8.37x10^{7} \pm 1.27x10^{7}$d
F0	5.52 (5.00 - 6.01)	10.17 (9.17 - 11.60)	$3.01x10^{10} \pm 1.62x10^{10}$a

F0= unformulated conidia; F1= kaolin clay based formulation; F2= maize flour based formulation ; F3= banana flour based formulation;F4 = cassava starch based formulation.

III.1.5.4. Effect of mycoinsecticide formulation

in the green-house conditions

III.1.5.4.1. Effect of mycoinsecticides formulations on plant growth parameters

Results of growth parameters from the greenhouse trial showed that 8 weeks after infestation (WAI) with adult weevils (corresponding to 16weeks after planting, WAP), plant height, pseudo-stem diameter, the average number of functional leaves, and leaf area were significantly different between infested and un-infested plants with respectively 39.80cm, 24.20 cm, 3.8 and 2085.98 cm^2 for infested plants and 43.50 cm, 27.50 cm, 6.75 and 2960.07 cm^2 for non-infested ones (table XIX).

The results also showed the positive effect of formulations on plant growth (table XIX). In general, the majority of *Beauveria bassiana* based formulations showed a good effect on growth parameters six weeks after exposure to treatments. Analysis of variance revealed that means of infested-treated plant height (with different formulations) was high compare to control (T0-) and those that received treatment T1'. As far as the number of functional leaves was concerned, treatments T0+, T1', T4' et T4 distinguished themselves with higher values than control T0- and other treatments. The highest value of the foliar area was obtained for plants treated with T4 (2749.76 cm^2) and T0+ which were not significantly different from all other treatments except T1.

Table XIX. Growth of banana plant 16WAP in the screenhouse

Application method	Treatment	Plant height (cm)	Pseudotronc ϕ (cm)	functional leaf number	Leaf area (cm^2)
Applic 1	T1	41.65 ± 2.56ab	25.00 ± 0.97a	3.00 ± 0.76c	1412.08 ± 485.98b
	T2	42.47 ± 0.76ab	22.57 ± 0.30a	3.57 ± 0.53bc	1752.00 ± 260.55ab
	T3	43.30 ± 1.74ab	24.20 ± 1.28a	3.40 ± 0.24bc	1582.74 ± 190.61ab
	T4	45.86 ± 2.34ab	25.40 ± 1.08a	5.60 ± 0.30ab	2749.76 ± 203.39a
Applic 2	T1'	38.15 ± 2.52b	22.60 ± 0.84a	4.80 ± 0.39abc	1875.39 ± 177.71ab
	T2'	47.12 ± 1.82ab	24.75 ± 1.16a	3.50 ± 0.38bc	2224.99 ± 329.39ab
	T3'	52.00 ± 0.5a	25.56 ± 0.85a	3.37 ± 0.41bc	1951.36 ± 275.28ab
	T4'	46.70 ± 1.94ab	25.00 ± 0.70a	5.00 ± 0.70abc	2588.25 ± 342.54ab
Control	T0+	43.50 ± 5.81ab	27.50 ± 3.23a	6.75 ± 0.48a	2960.07 ± 31.09a
	T0-	39.80 ± 1.85b	24.20 ± 1.16a	3.8 ± 0.86bc	2085.98 ± 528.79ab

Applic 1=post-infestation; Applic2 = pre-infestation; T1' and T1= plants infested and treated with *B. bassiana* formulation based on kaolin; T2' andT2 = plants infested and treated with *B. bassiana* formulation based on Maize flour; T3' and T3 = plants infested and treated with *B. bassiana* formulation based on banana flour; T4' and T4= plants infested and treated with *B. bassiana* formulation based on cassava starch; T0- = plants infested and untreated; T0+ =

plants non-infested and untreated; Means followed by same letter in the same column are not significantly different with the Tukeys HSD test, $\alpha = 0.05$;

III.1.5.4.2. Effect of formulations on immature weevils and plants damages

The population of immature life stage (eggs, larvae, and pupae) reduced considerably with the pre-infestation (13.25 insects) method compare to the post-infestation method (26 insects) and control (21 insects). The numbers of immature *C. sordidus* were 16 - 38, 6 - 22, and 21 respectively for post-infestation (Applic1), pre-infestation (Applic2), and negative control (T0-). The lower number of immature weevils were achieved when plants were treated with cassava-based formulation, T4 (16 insects) followed by T1 (19 insects) using the post-infestation method. Treatment T4' (6 insects) followed by T3' and T2' showed a low number of immature weevils when applied before infestation (table XX).

Table XX. Effect of mycoinsecticide formulations on immature stages in greenhouse conditions

Application method	Treatment	Total immature	Living per stage			Mortality stage			Mycosis per stage		
			E	L	P	E	L	P	E	L	P
Applic1	T1	19	4	12	2	0	0	0	0	0	0
	T2	31	16	10	1	0	1	0	0	1	0
	T3	38	5	18	7	0	0	6	0	0	0
	T4	16	6	8	1	1	0	0	0	0	0
Applic2	T1'	22	0	13	4	2	0	0	0	0	0
	T2'	14	0	10	4	0	0	0	0	0	0
	T3'	11	0	7	1	0	0	0	0	0	0
	T4'	6	0	3	3	0	0	0	0	0	0
Control	T0-	21	0	19	2	0	0	0	0	0	0
	T0+	0	0	0	0	0	0	0	0	0	0

Applic 1 post-infestation; Applic 2: pre-infestation; E: egg; L: larva; P: pupa; T1' and T1: plants infested and treated with *B. bassiana* formulation based on Kaolin; T2' and T2 : plants infested and treated with *B. bassiana* formulation based on Maize flour; T3' and T3 : plants infested and treated with *B. bassiana* formulation based on banana flour; T4' et T4: plants infested and treated with *B. bassiana* formulation based on cassava starch; T0- : plants infested and untreated; T0+ : plants non-infested and untreated.

Plants with the youngest yellow or dead leaf were considered as attacked by *C. sordidus*. Almost all treatments had infested plants except T4'. The Incidence was moderate (≤ 50 % incidence) for all treatments except negative control and T2 with respectively 60 and 70 % plants showing symptoms (Table XXI). Un-infested plants did not show symptoms (T0+). The lowest

incidence for Applic1 was achieved with treatments T4 (10 %), T3 (10 %) followed by T1 (40 %). As far as Applic2 was concerned, the values of incidence lower than the negative control (T0-) were obtained for treatments T4' (0 %) followed by T2' (20 %) and T1' (30 %).

In general, damages observed on corms treated with formulations were low than control except for T3 and T4' with high damages respectively on the central cylinder and cortex (Table XXI). Data of damages collected 16 WAP indicated that percentage damage of cortex and central cylinder were 35.50 and 34.50 %, 8 - 25.50 % and 1.50 - 52 %, 14 - 46 % and 7.50 - 26 % respectively for negative control (T0-), Applic1 and Applic2. The values of the severity of damage was lower for T4 (1.5 % central cylinder damage and 8 % cortex damage) followed by T2 and T1 for Applic1 and T1' (7.5 % central cylinder damage and 14 % cortex damage) followed by T2' and T3'for Applic2 (Table XXI).

Table XXI. Incidence and severity of infestation according to biopesticide implementation methods, 16 weeks after planting

Application method	Treatment	Incidence	Severity		Reduction	
			CC (%)	Cortex (%)	CC (%)	Cortex (%)
Applic1	T1	40	25	21.5	27.54	39.44
	T2	70	23	10	33.33	71.83
	T3	10	52	25.5	-50.72	28.17
	T4	10	1.5	8	95.65	77.46
Applic2	T1'	30	7.5	14	78.26	60.56
	T2'	20	23	26.5	33.33	25.35
	T3'	50	25.5	27	26.08	23.94
	T4'	0	26	46	24.64	-29.58
Control-	T0-	60	34.5	35.5	-	-
Control+	T0+	0	0	0		*

Applic 1=post-infestation; Applic2 = pre-infestation; T1' and T1= plants infested and treated with *B. bassiana* formulation based on Kaolin; T2' andT2 = plants infested and treated with *B. bassiana* formulation based on Maize flour; T3' and T3 = plants infested and treated with *B. bassiana* formulation based on banana flour; T4' and T4= plants infested and treated with *B. bassiana* formulation based on cassava starch; T0- = plants infested and untreated; T0+ = plants non-infested and untreated.

III.1.5.4.3. Insecticidal potential of biopesticides formulations against *C. sordidus* and conidia persistence

The results showed that temperature during trial was ambient (23.42 - 25.91 ◦C) and moisture 0.08 - 0.15 (fig.37). All formulations were found pathogenic to adult weevils in greenhouse conditions (Appendix 4). Mortality rates were significantly affected by time ($F = 155.1$; $df = 5$; $P < 0.0001$). Six weeks post-treatment, mortality rates were significantly different between application method and negative control ($F = 16.50$; $df = 2$; $P = 0.001$).

However, mortality did not differ significantly between pre-infestation (53.33 - 68.67 %) and post-infestation (66 - 84.67 %) methods respectively Applic1 and Applic2. Fungal outgrowth observed on dead insects confirmed that fungi *B. bassiana* was responsible for weevil's death (Appendix 5). However, there was a significant difference in infection rates between treatments ($F = 14.06$; $df = 8$; $P = 0.009$) at the end of the experiment. Treatment T4 based on Cassava formulation caused the highest mortality (84.67 %) and infection (75.33 %) with the post-infestation method followed by treatment T1, based on kaolin formulation (T1) which showed high virulent for both post-infestation (76 % mortality and 72 % mycosis) and pre-infestation method (68.67 % mortality and 66 % infection). Average mortality in control was 12 % but no fungus outgrowth was observed on dead insects.

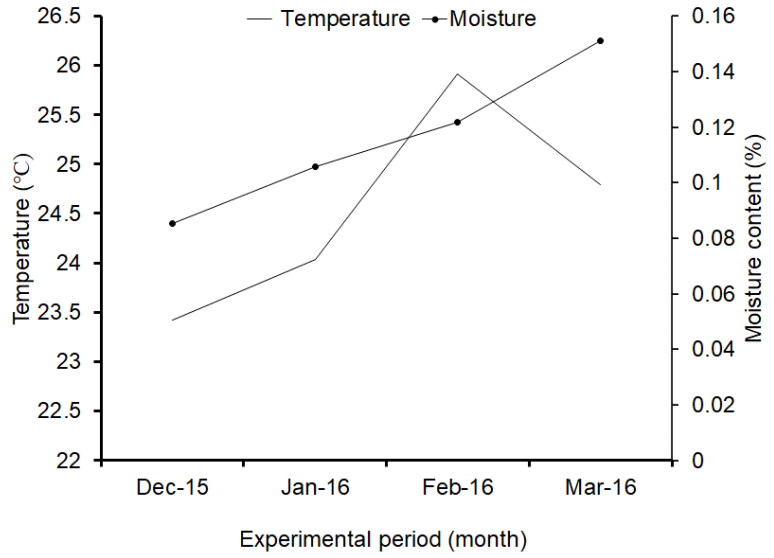

Fig. 34. Variation of temperature in the screenhouse and water content of the soil in pots during bioassay.

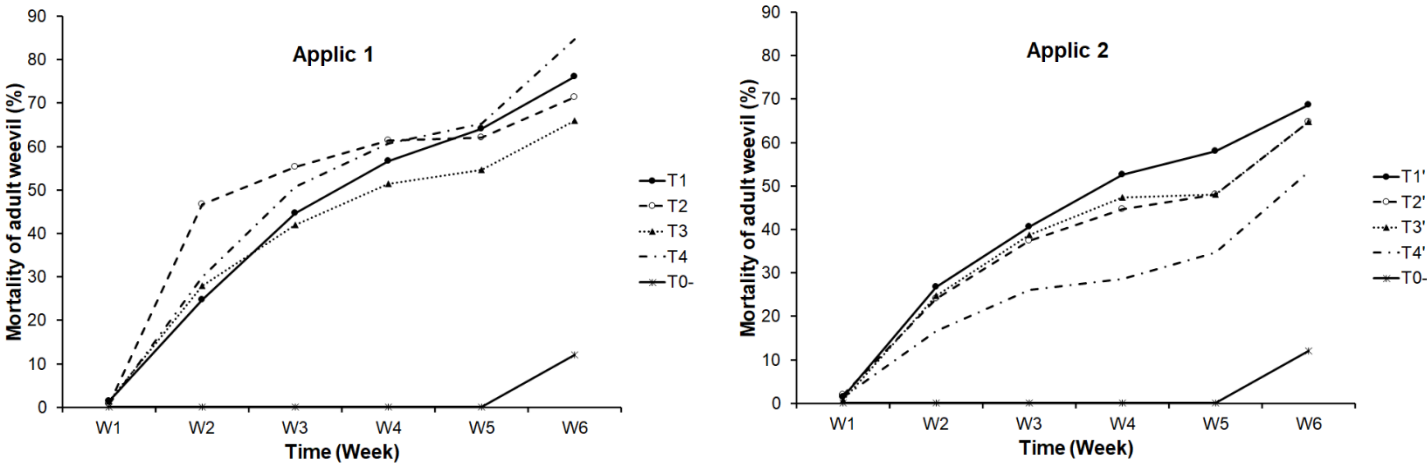

Fig. 35. Cumulative mortality six weeks after biopesticides implementation in the screenhouse.

T1' and T1= plants infested and treated with *B. bassiana* formulation based on Kaolin; T2' and T2 = plants infested and treated with *B. bassiana* formulation based on Maize flour; T3' and T3 = plants infested and treated with *B. bassiana* formulation based on banana flour; T4' and T4= plants infested and treated with *B. bassiana* formulation based on cassava starch; T0 = plants infested and untreated; Applic 1=post-infestation method; Applic2 = pre-infestation method .

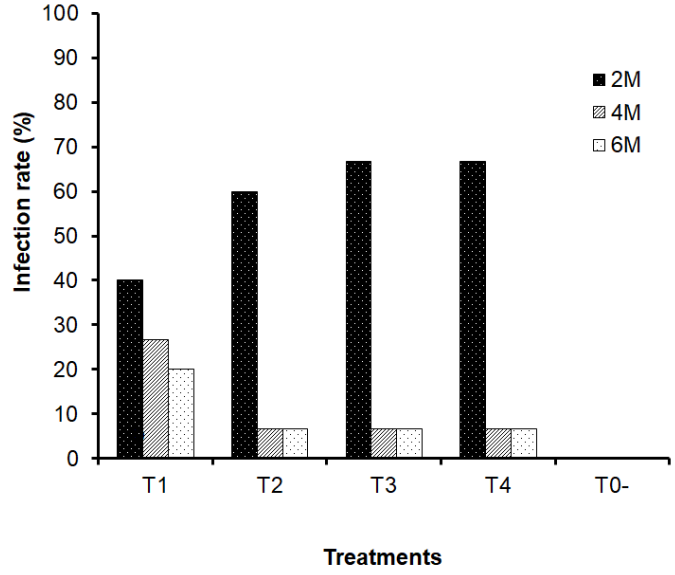

Fig. 36. Conidia survival 6 months after application in the greenhouse.

T1= plants infested and treated with *B. bassiana* formulation based on Kaolin; T2 = plants infested and treated with *B. bassiana* formulation based on Maize flour; T3 = plants infested and treated with *B. bassiana* formulation based on banana flour; T4= plants infested and treated with *B. bassiana* formulation based on cassava starch; T0- = plants infested and untreated; 2M= two months; 4M= four months; 6M= six months

Conidia persisted in the soil for more than 6 months (fig. 38). Mortality of adult weevil reduced with time. The infectious potential of treatments T2 (60 – 6 %), T3 (66.67 - 6.67 %), T4 (66.67 - 6.67 %), based respectively on maize, banana, and cassava flour, decreased rapidly between 2nd month and 6th month compared to the kaolin-based formulation, T1 (40 - 20 %) (fig. 36, 37 and 38).

III.1.5.5. Effect of formulation applied alone in the field

III.1.5.5.1. Effect of growth parameters

Results on growth parameters fifteen months after planting (15MAP), period corresponding to plants flowering or 3 months after application of formulations (3MAA) showed no significant difference on plant height ($\chi2 = 3.89$; df = 2; P = 0.14) and number of functional leaves between treatments ($\chi2 = 0.47$; df = 2; P = 0.79). Plant girth was the only growth parameter which varied between treatments ($\chi2 = 7.77$; df = 2; P = 0.02) and treatment T1 had the lowest girth (59.91 cm) compare to T0 (64.24 cm) and T2 (65.43 cm) (Table XXII).

Table XXII. Plant growth parameters 15 MAP in the field

Treatments	Height (cm)	Stem girth (cm)	Functional leaf number
T0	300.70 ± 12.80	64.24 ± 2.23	8.12 ± 0.33
T1	289.50 ± 8.90	59.91 ± 1.61	7.81 ± 0.30
T2	308.62 ± 7.11	65.43 ± 1.02	7.94 ± 0.28
	$\chi2$= 3.89, df = 2, P= 0.14	$\chi2$= 7.77, df = 2, P < 0.02	$\chi2$=0.47, df = 2, P = 0.79

T1= plants treated with *B. bassiana* formulation based on Kaolin; T2 = plants treated with *B. bassiana* formulation based on cassava starch; T0 = plants untreated.

III.1.5.5.2. Evaluation of weevil population per month

During the 25 months of the study,1926 weevils were caught in the field and the highest catches were recorded in treatment T1 (852 weevils) compare to T0 (488 weevils) and T2 (586 weevils). The curve presented in figure 39xxx showed a total reduction of catches weevil the 2 months (May 2016 and June 2016) next to the first application (6 MAP) even in the control plot. After these two months, the number of capture weevil increase for 5 months. At the 2nd application

(12MAP), the population of weevil reduces slowly in all treatments except treatment T1 where kaolin-based formulation was applied. Results of trapping after the 3rd application (22MAP) revealed that the weevil population state reducing directly the next month (September 2017) for treatment T1 and T2 while in treatment T0 the population increase then decrease. Treatment T2 showed a relatively low population compared with the control, during May 2016, September-December 2016, February 2017, and December-April 2018 (fig. 39A).

In general, within the 25 months of evaluation of biopesticide performance in the field, the population fluctuated much, temperature, relative humidity, and rainfall were ranged 22.43 - 26 ◦C,74.54 - 89.91 %, and 0 - 15.12 mm respectively (fig. 39B). However, the fluctuation was not related to any of the environmental factors monitored. No correlations were detected between the weevil population and agroecological parameters: temperature, humidity, and rainfall for all treatments (table XXIII). Meaning that these factors did not affect population fluctuation for different treatments.

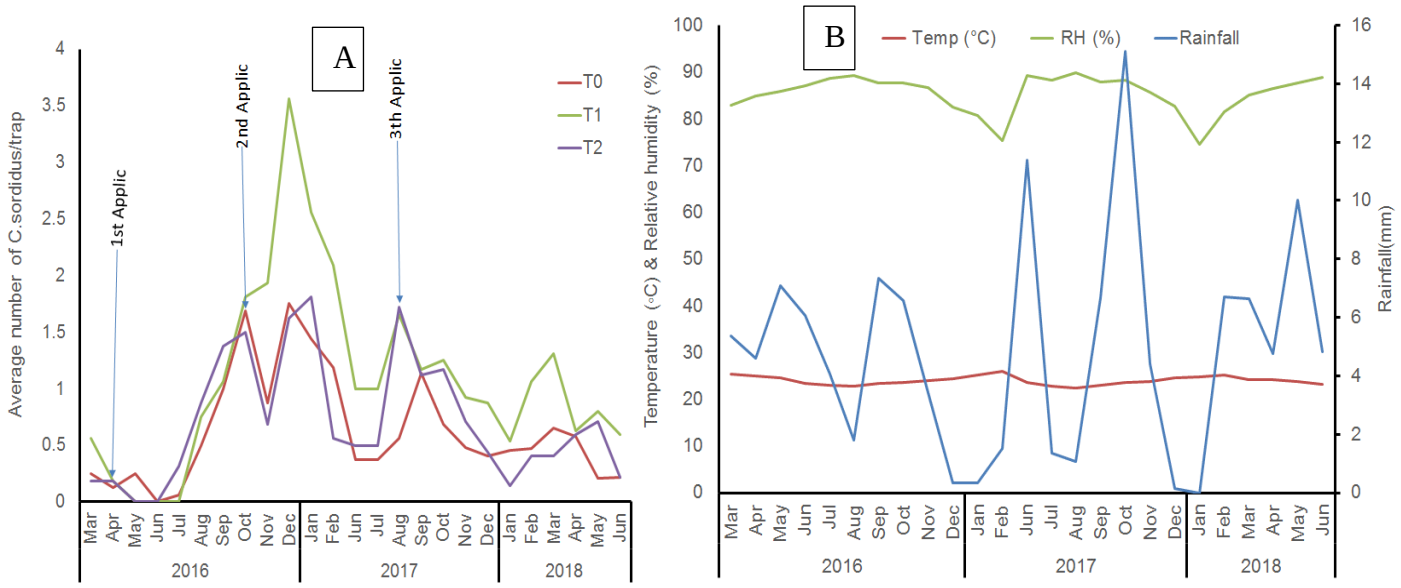

Fig. 39. Climatic variation (B) and fluctuation of weevil population during the field experiment (A) T1 = plants treated with B. bassiana formulation based on Kaolin; T2 = plants treated with B. bassiana formulation based on cassava starch; T0 = plants untreated.

Table XXIII. Correlation between weevil population and environmental factors

Treatments	Rainfall	Temperature	Relative humidity
T0	R = -0.19; P = 0.35;	R = 0.16; P=0.44	R = 0.24; P=0.24
T1	R =-0.28; P = 0.17	R = 0.19; P=0.36	R = 0.25; P=0.22
T2	R =-0.09; P = 0.67	R = 0.24; P=0.24	R= 0.15; P=0.46

T1= plants treated with *B. bassiana* formulation based on kaolin; T2 = plants treated with *B. bassiana* formulation based on cassava starch; T0 = plants untreated

It is important to mention that dead weevil showing fungi out growth was observed on plots that received T1 and T2 under pseudo-stem trap (Appendix).

III.1.5.5.3. Effect of treatments on damages and yield

No plants showed external symptoms of banana weevil infestation by falling, but while trimming the corm and evaluating internal symptoms; cortex and center cylinder was infested for all treatments. The diameter of cortex and center cylinder were ranged between 26 - 30 cm and 12.86-14.50 cm respectively but did not differ between treatments ($P > 0.05$). The mean damage level was between 4.30 - 10 % (Table XXIV). Kruskal Wallis test revealed that no significant difference between treatments for central cylinder damages ($\chi2 = 0.12$; $df = 2$; $P = 0.938$) and cortex damages ($\chi2 = 0.127$; $df = 2$; $P = 0.942$).

The percentage of plants showing damages depended on damages level for center cylinder ($\chi2 = 8.76$; $df = 2$; $P = 0.03$) and cortex ($\chi2 = 9.598$; $df = 2$; $P = 0.02$), (Table XXV). The high rate of plants showed low-level damage (LD) of the cortex for treatment T0 (66.67 %) and T2 (62.5 %) while treatment T1 (64.28 %) showed a high rate of plant damage at a moderate level (table XXV). More than 14 % of plants treated with kaolin clay (T1) were heavily damaged. As for the central cylinder, all treatments had a high rate of plants with low damage (50 - 83.33 %). Treatment T1 had a high percentage of plants showing moderate level damage (42.86 %). Treatment T0 had 16.67 % of plants showing very heavy damage.

At the first crop cycle, the average bunch weight was ranked between 13.13 - 15.8 kg. Statistical analysis with Anova revealed that bunch weight was not significantly different between treatments ($F = 0.79$; $df = 2$; $P = 0.46$).

Table XXIV. Damages due to banana weevil by treatment and corm diameter

Treatments	Bunch weight (kg)	Diameter (cm)		Damage (%)	
		C	Cc	C	Cc
T0	14.60 ± 1.52	27.13 ± 0.83	14.50 ± 0.50	5.83 ± 2.12	5.00 ± 2.75
T1	13.13 ± 1.20	30.00 ± 4.47	13.60 ± 0.60	10.00 ± 1.82	7.33 ± 1.70
T2	15.80 ± 1.84	26.07 ± 1.30	12.84 ± 0.52	5.71 ± 1.80	4.30 ± 1.27

C=cortex; Cc= central cylinder; T1= plants treated with *B. bassiana* formulation based on kaolin; T2 = plants treated with *B. bassiana* formulation based on cassava starch; T0 = plants untreated.

III.1.5.5.4 Effect on non-target insects and conidia persistence

In addition to *Cosmopolites sordidus*, other species of insects including *Pollytus mellerborgi* and unidentified species showed *Beauveria bassiana* outgrowth (fig. 40).

Soil samples collected eight months after 2nd application (8MA2A) in the field 1 were evaluated by releasing 2 - 3 insects per sample. The results showed that conidia were still viable in the field after 8 months. Treatment T1 caused mortality 2 times higher than treatment T2. No insect showed fungi outgrowth from the control soil sample (fig.41).

Table XXV. Percentage of plants showing internal symptoms by damages level

Plant part	Damage of plants per level (%)			
	LD	MD	HD	VHD
		T0		
Cortex	66.67	25	0	8.33
Centre cylinder	83.33	0	0	16.67
		T1		
Cortex	21.43	64.28	0	14.28
Centre cylinder	50	42.86	0	7.14
		T2		
Cortex	62.5	31.25	0	6.25
Centre cylinder	75	41.67	0	8.33

LD = low level (0 - 5 %); MD= moderate damage (> 5 - 10 %); HD= high damage (> 10 - 15 %)); VHD=very high damage (>15 %); T1= plants treated with *B. bassiana* formulation based on Kaolin; T2 = plants treated with *B. bassiana* formulation based on cassava starch; T0 = plants untreated.

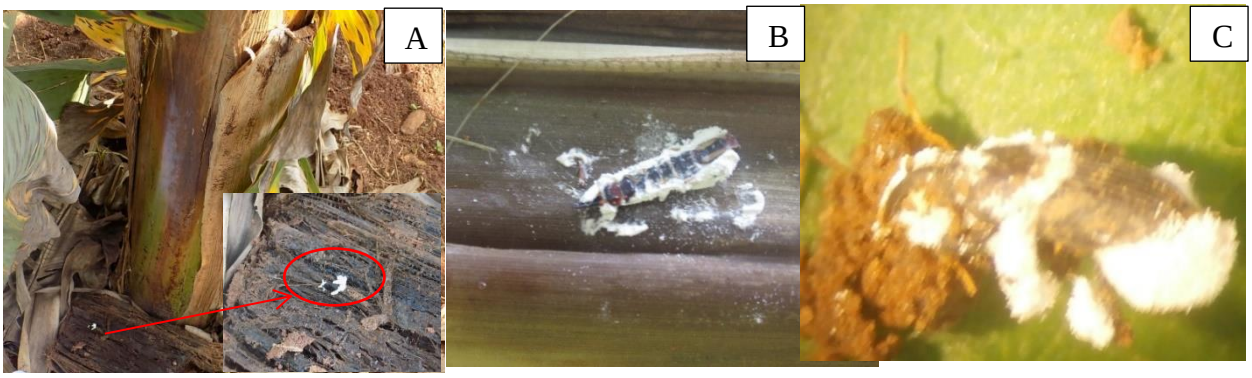

Fig. 37. Target and non-target insects infected in the field: A) *C. sordidus*; B) unidentified insect; C) *Pollytus mellerborgi*.

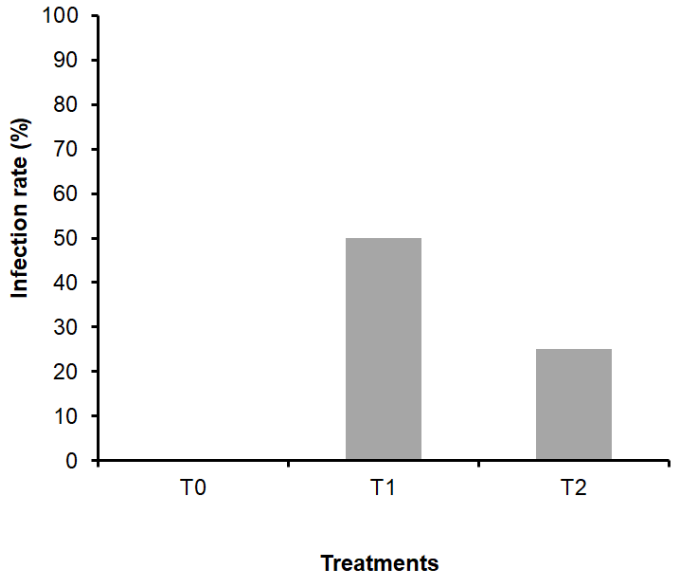

Fig. 38. Infection rate of weevil from soil sample collected 8 months after application (8MAA) of biopesticide in the field. T1= plants treated with *B. bassiana* formulation based on Kaolin; T2 = plants treated with *B. bassiana* formulation based on cassava starch; T0 = plants untreated.

III.1.5.6 Formulation performance combined with pheromone

III.1.5.6.1 Attractiveness of traps

For the 28 days of traps evaluation Pheromone associated pseudo-stem trap caught almost 17 times weevils (254 weevils) compare to pseudo-stem trap (15 weevils). The results revealed that attractiveness of weevil depended on treatments ($t = -9.66$; $df = 46$; $P < 0.001$), exposure time

(df = 3; F = 6.96; P = 0.001) and (df = 3; F = 3.02; P = 0.04). The number of *C. sordidus* caught per trap was significantly high at from 7- 21 days post exposure for both treatments (fig. 42).

III.1.5.6.2 Insecticidal potential of formulation combined with pheromone

One-day post-application, insects were already present under the trap, and more than 95 % of weevils' present were in contact with formulation. Mortality rates were 0 – 83.33 % with significantly high rate where formulation was applied (t = -3.13, df = 34, P = 0.0035) (fig.43).

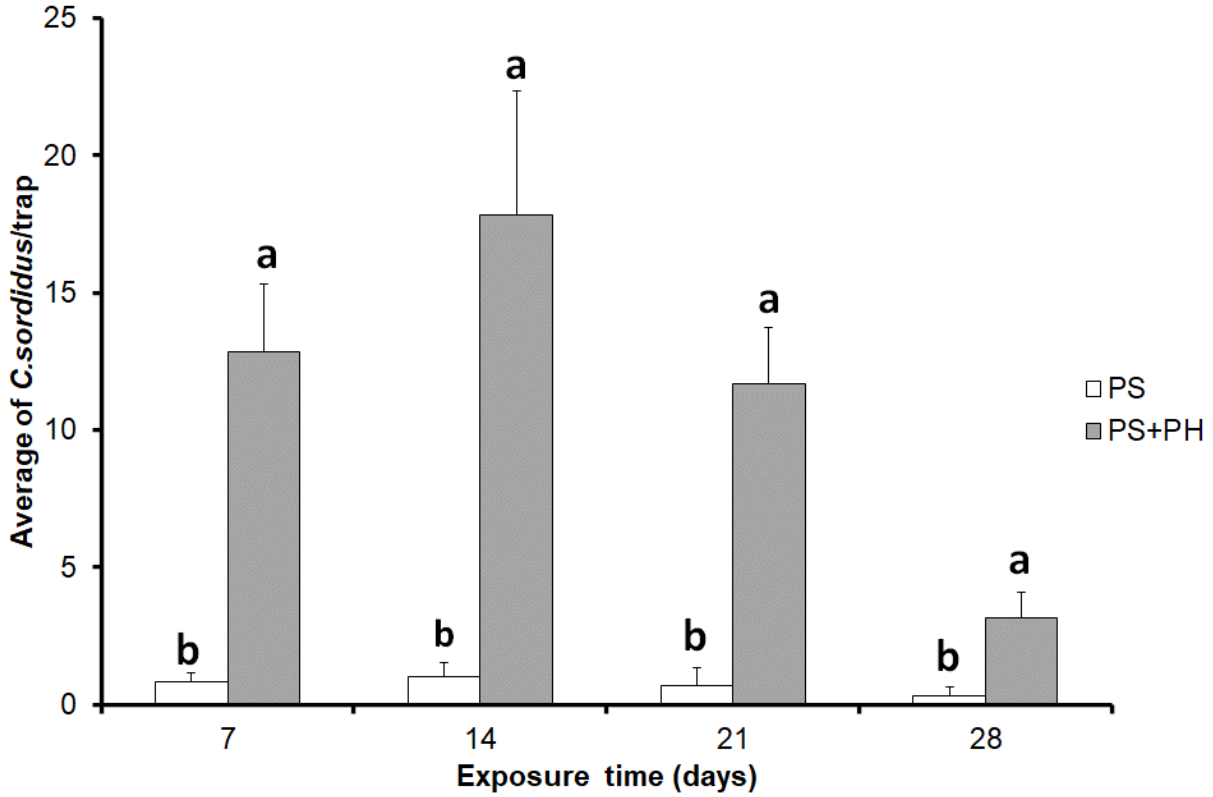

Fig. 39. Attractiveness of pheromone baited traps compared to pseudo-stem traps. Means followed by same letter in the same exposure time are not significantly different with the Tukeys HSD test, α = 0.05; PS= Pseudostem trap; PS+PH = pseudostem trap associated with pheromone.

Data showed that mortality rates were also affected by exposure time and interaction exposure time X treatments (Exposure time: F = 14.94; df = 2; P < 0.001; Interaction: F = 14.94. df = 2; P < 0.001). Mycosis rates range 0 – 78.70 %, presented the same trend as mortality rates with significant difference between treatments (t = -3.24, df = 34, P = 0.0026), exposure time (F = 6.61; df = 2; P = 0.004) and interaction exposure time X treatment(F = 6.61; df = 2; P = 0.004). Mycosis rate was high where formulation was applied.

Other insect species such as ants (*Pachycondyla tarsata*), stem borer (*Metamacius hemipterus*), borer (*Polytus mellerborgi*) and others coleopteran (unidentified) were also found dead due to *Beauveria bassiana* on application point (Appendix 7).

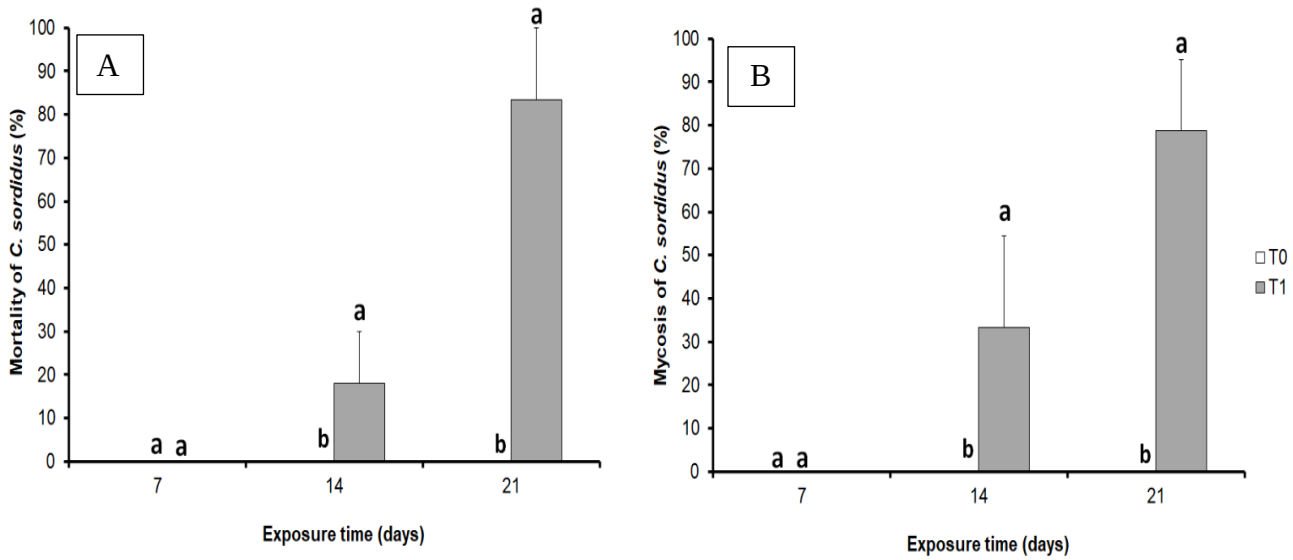

Fig. 40. Effect of treatments on the weevil population in the field. T0=control, T1=formulation: A= total population caught; B= Adult Mortality rates; C= Mycosis rate of *C. sordidus*; Means followed by same letter in the same exposure time are not significantly different with the Tukeys HSD test, $\alpha = 0.05$;

III.2. Discussion

Our study showed that the fungal isolates were pathogenic and virulent to tested stages of *C. sordidus*. However, the performance of isolates depended on life stages. Out of the six isolates studied, four and five were highly pathogenic on larva and pupa respectively. Unlike *M. anisopliae* where three isolates tested were highly virulent to all life stages, *B. bassiana* isolates' virulence varied significantly, with BIITAC10.3.3 being the only highly pathogenic isolate against larva and BIITAC6.2.2 and BIITAC8.1.5 for the pupa. The difference in response between *C. sordidus* life stages could be due to the composition of the insect cuticle that varied with life stages. The rigid shell of eggs may have acted as a barrier to fungi penetration causing low infection of eggs. Moreover, at a certain life stage, some insect exudes glandular secretions which display antifungal activity against entomopathogenic fungi (Ortiz-Urquiza and Keyhani, 2013). The high infectivity of fungal isolates studied is of great importance given that the larval stage is the most damaging life stage of banana weevil (Gold and Messiaen, 2000). We hypothesize that a successful infection

process confirmed in larvae in the banana corm will most likely result in a wider spread of the disease within the corm, coupled with the faster halting of damage to the plant compared to the mortality of the adult stage alone in case fungi are endophyte. Nganso *et al.* (2010) found that *B. bassiana* strain GHA (Botanigard) applied on corm in laboratory condition in Cameroon was not effective on adult, eggs, and larva of *C. sordidus* while Kaaya *et al.* (1993) reported high susceptibility of both larval and adult stages of *C. sordidus* to Kenyan, Thailand and UK isolates of *B. bassiana* and *M. anisopliae* causing 63 to 97 % mortalities. while studying the effect of Ugandan and Kenyan isolates of *B. bassiana* on eggs, larvae, and adult *C. sordidus* also reported high infectivity on larvae causing 60 % mortality. Similarly, to our findings, Kaaya *et al* (1993) and Godonou (1999) reported that larvae were highly susceptible. This is however the first report of virulence of entomopathogenic fungi against *C. sordidus* pupae.

The pathogenicity and virulence of the different isolates tested were significantly different. This denotes differences between isolates belonging to the same species as well as the difference between both species. Belonging to the same fungal species is therefore not a guarantee for similar virulence levels since the difference between isolates can rely on different types and quantity of enzymes and metabolites (which are important virulence determinants) produced by specific strain (Abdelaziz *et al.* 2018). Similar results have been reported by Ruelas-Ayala *et al.* (2013), Cheng *et al.* (2016), and Yasin *et al.* (2017) using *B. bassiana* and *M. anisopliae* isolate against other weevils like *Tenebrio molitor* (L.; Curculionidae: Coleoptera), *Sitophilus zeamais* (Motschulsky; Curculionidae: Coleoptera), *Curculio nucum* (L.; Curculionidae: Coleoptera) and *R. ferrugineus* respectively. The six virulent indigenous isolates in the present study against the banana root borer were found effective against adult weevil (Membang, 2013). Recently, these six isolates were reported highly effective against cocoa mirid *Sahlbergella singularis* (Haglun; Hemiptera: Miridae) (Mahot *et al.*, 2019). Both *M. anisopliae* and *B. bassiana* are known to be infectious to host of numerous insect orders including Orthopera, Lepidoptera, Dermaptera, Diptera, and Coleoptera. Further studies are warranted to assess the range of pests that could be controlled while using each of the indigenous Cameroonian isolates.

Our investigation on pathogenicity on the immature life stage of *C. sordidus* also revealed that despite the regular change of substrate (mashed corm) in eggs and pupae bioassays, Cameroonian isolates could favorite disease transmission from on life stages to another. This horizontal transmission contributed to high combined mortality and infection that varied among

isolates. It was pronounced in the three *M. anisopliae* isolates while only one *B. bassiana* isolate resulted in horizontal transmission. This is the first such report of horizontal transmission of entomopathogenic fungi within the immature stages of *C. sordidus*. This means that conidia of primary inoculum applied during artificial infection remains viable during metamorphose of banana weevil which belongs to the holometabolan group. The holometabolan group characterizes by changing physical morphology completely during their life cycle. Therefore insect molting has not prevented the infection process. Horizontal transmission is an added value since it adds to the mortality of the directly inoculated stage, and subsequently increasing total mortality. Further studies are warranted to understand the process and/or characteristics underlying the differences between isolate, particularly differences between *M. anisopliae* and *B. bassiana* in their potential to horizontally infect banana weevil. Our findings are however similar to those reported by Gindin *et al.* (2006) in which *M. anisoplie* killed horizontally neonate larva of *R. ferrugineus* when eggs were treated; and Asi *et al.* (2013) where *B. bassiana* and *M. anisopliae* reduced emergence of adult *Spodoptera litura* (Fabricius; Lepidoptera: Noctuidae) when the pupal stage was treated.

Leaves and corm of banana plantains (variety Ebang) were not colonized by either Beauveria or Metarhizium isolates. The absence of fungi colonization may be related to the methodology and variety used. Akello *et al.* (2007) in her study on *Beauveria bassiana* (Balsamo) Vuillemin as an endophyte in tissue culture banana (*Musa* spp.) showed fungus establishment in plants depend on inoculation method with the dipping of tissue culture plant in inoculum as the best method. Akutse et *al.* (2013) reported that host plant species and species varieties (cultivars) can influence the endophytic abilities of the specific isolate. Their findings also revealed that a fungus can colonize a part of a plant but not another.

Our results on the thermal response of Cameroonian fungal isolate revealed that EPFs studied exhibited remarkable adaptability reflected by their ability to germinate, grow and produce conidia at a wide thermal tolerance range with fungal activity inhibited at 37 °C. However, differences in thermal responses varied depending on biological traits. The temperatures 13 and 33 °C that were tolerated for germination and mycelia growth by MIITAC11.3.4 and MIITAC6.4.2, were found detrimental for the conidia production of the two isolates. This suggests that conidiogenesis is highly sensitive steps that require special attention given that it also affects auto-dissemination of the fungus and consequently its persistence in the field which in return will affect the cost of industrialization for virulent strain. Conidiogenesis of fungi depends on

reproductive mechanisms that are controlled by environmental, nutritional, genetic, and hormonal factors (Zimmermann, 2007). Our findings are closer to those of Tefera and Pringle (2003), who found that the optimum temperature for germination was wider for both *B. bassiana and M. anisopliae* (20 and 30 °C), while the optimum temperature for growth and sporulation was more restricted to 25-30 °C and 20-25 °C for *B. bassiana and M. anisopliae* respectively.

We observed that conidia production of *B. bassiana* isolates was 12 times higher compared with *M. anisopliae* isolates. The PDA media could be more suitable *B. bassiana* conidiogenesis. Similarly, Lopes *et al* (2013), while studying the diversity of indigenous *B. bassiana* and *Metarhizium* spp. in a commercial banana field of Brazil and their virulence toward *C. sordidus*, found that, *B. bassiana* produced between 5 and 95 times more conidia per colonized host than indigenous *Metarhizium* spp. isolates. Gandarilla-Pacheco *et al.* (2012) also reported high sporulation of *B. bassiana* strains at almost all tested temperatures while evaluating conidia production and mycelial growth in solid culture media for native strains of entomopathogenic fungi isolated from citrus-growing areas of México. Other authors also examined conidia productivity on culture media and reported variation in conidial yield as affected by species and strains (Safavi et *al.*, 2007).

Additionally, the thermal response (tolerance and optimum ranges) was fungus isolate-dependent with most isolates tolerating 13-33 °C and MIITAC11.3.4 MIITAC6.4.2 which had narrow tolerance ranges, 13-29 °C and 15-33 °C respectively. The optimum temperature range for maximum conidia germination was widest (20-33 °C) for the three *M. anisopliae* isolates, followed by two *B. bassiana* isolates BIITAC8.1.5 and BIITAC6.2.2 with respectively 20-25 °C and 25-29 °C which had the same width but different limits. The trend of mycelia growth was specific to each fungus with high mycelial growth between 13-29 °C, 15-29 °C, 20 °C and 25 °C, except BIITAC6.2.2 and MIITAC11.3.4 which had the same optimum thermal range, 20-29 °C. Four of the six isolates showed a wide optimum thermal range for conidia production, 13-25 °C, and 15-25 °C for two *B. bassiana* isolates (BIITAC10.3.3 and BIITAC8.1.5), and 13-29 °C and 15-25 °C for two *M. anisopliae* isolates (MIITAC6.2.2 and MIITAC11.3.4 respectively). However, one *M. anisopliae* isolate (MIITAC6.4.2) showed high conidia production at a specific temperature (25 °C) while two isolates (BIITAC8.1.5 and MIITAC11.3.4) of both fungi species (*B. bassiana* and *M. anisopliae)* had the same optimum thermal ranges, 15-25 °C. The difference in the thermal response of fungal isolates may depend on isolates origin (Vidal *et al.*,1997; Fargues *et al.*, 1997;

Teja and Rahaman, 2016; Alali *et al.*, 2019). Augustyniuk-Kram and Kram (2012) highlighted that EPF strains from forest grow better at low temperatures compared with strains from agricultural fields that tolerate high temperatures. However, the optimum temperature ranges for germination and sporulation obtained in this study were favorable for the growth of banana crop (19-30 °C), development of BRB (thermal tolerance 12 °C to less than 34 °C, optimum ranges 20-30 °C) and the development of fungus infection in several hosts including BRB (Cuillé, 1950; Lopes *et al*., 2011; Membang *et al.*, 2020). Additionally, all isolates exhibited high activity at 25 °C which is the optimal temperature for adult banana root borer activity, favorable for disease occurrence (Cuillé, 1950; Lopes *et al.*, 2011*)*. Meaning that these fungi can remain active in the banana rhizosphere. The outcome of our result is important for the dissemination of inoculum in the host through direct contamination, indirect contamination, and secondary transmission of conidia (Vega *et al.*, 2007), exploited for effective control of insect pest using attractants (Tinzaara *et al.*, 2015; Opisa *et al.*, 2019).

Most isolates in this study were from a forest-savanna transition zone, the microclimate and microhabitat may influence the adaptation potential of a fungus as well as genetics and molecular regulation that determine the expression or repression of morphological features (Papagianni, 2004; Abdelaziz *et al.*, 2018). Similarly, many authors showed that differences in thermal response (Tefera and Pringle, 2003; Borisade and Magan, 2014; Ahmad *et al.*, 2016; Alali *et al.*, 2019; Heviefo *et al.*, 2019). Alali *et al.* (2019) found that colonies of five *B. bassiana* isolates from hot environments in Syria grew at 20–30 °C but not at 35 °C. Ahmad *et al.* (2016) confirmed inhibition of germination, growth, and conidia production at 35 °C for two isolates of *B. bassiana* from Syria which tolerated 15–30 °C and obtained optimum fungal development (mycelia growth, conidiation, and germination) in the range of 25–30 °C. In the same lines, Heviefo *et al* (2019) showed that 35 °C was detrimental for the growth of a *B. bassiana* isolate from Benin which tolerated 20-30 °C with highest growth and sporulation at 15-30 °C and 25 °C respectively. Instead, Borisade and Magan (2014) showed that EPF from Benin and the United Kingdom had wide thermal profiles; *B. bassiana* grew at 25–35 °C with optimum growth and sporulation ranges between 25-30 °C while *M. anisopliae* grew and sporulated at 25–37 °C with 25–30 °C as an optimum thermal range. Borisade and Magan (2014) also demonstrated that the interval of thermal tolerance for *B. bassiana* and *M. anisopliae* isolates from a specific area can vary highly. Similar to our findings from Cameroon, a report on the thermal sensitivity of five Ethiopian EPF isolates,

one *B. bassiana*, and four *M. anisopliae*, revealed that *B. bassiana* tolerated 15-30 °C whereas *Metarhizium* spp. isolates germinated, grew, and sporulated between 15-35 °C (Tefera and Pringle, 2003). The slight difference can depend broadly on fungal origin since the isolates used in our study were from a forest-savanna transition zone characterized by Equatorial transition climate, hot and humid Equatorial climate with large temperature fluctuations 12-33 °C (IRAD, 2008). It is obvious that thermal tolerance ranges depend on the range of temperatures investigated. That is why Ahmad *et al* (2016) even suggested that thermal tolerance of EPFs should be considered as the main factor for the industrialization of products that can adapt to various environments. Our results also confirmed that *B. bassiana* and *M. anisopliae* are mostly mesophilic with optima temperature ranges between 20-30 °C as shown by Fargues *et al* (1997); Tefera and Pringle (2003).

Results on the epizootic potential of thermotolerant isolates revealed that mortality of non-inoculated insects increased with vector ratio, especially for the more virulent isolate BIITAC6.2.2 which caused up to 53 % mortality at a 50 %vector ratio. Two studies compared the pathogenicity of the six isolates included in our study (Mahot *et al.*, 2019 and Membang *et al.*, 2020). They found high virulence of BIITAC6.2.2, causing up to 90.0 ± 5.77 % and 96.7 ± 3.33 % mortality against the cocoa mirid *S. singulris* (Mahot *et al.*, 2019) and adult BRB (Membang *et al.*, 2020) respectively. These findings can explain the level of virulence on non-inoculated and therefore the potential of transmission of BIITAC6.2.2 . Ewald (1994) and Myers et *al.* (1995) reported that efficient transmission of disease by vectors is heavily associated with greater virulence. The high virulence of this isolate compared with others could be related to pathogenicity factors involved in fungus-host interaction. Pathogenicity factors such as destructive enzymes (protease, chitinase, and lipases) and metabolites can differ in type and amount depending on fungus species and strain (Butt *et al.*, 2016; Petrisor and Stoian, 2017; Abdelaziz et *al.*, 2018). Mortality of non-inoculated BRB in this study is high compare to Lopes *et al* (2011) who found 45 % mortality of non-inoculated BRB at vector ratio 50 % when evaluating horizontal transmission of *B. bassiana* Brazilian isolates. Contrary to Lopes et al (2011), food exchanges and removal of dead insects were done every 5 days for 35 days meaning that inoculum dissemination was only from primary sources (during inoculation) but not both as in the study of Lopes *et al* (2011).

A positive correlation was observed between the proportion of inoculated insects and infection of non-inoculated individuals. The inoculum was transferred from inoculated to non-inoculated insect and that living BRB are considered effective carriers of inoculum, contrary to

other coleopterans that detect and avoid entomopathogenic fungi (Zimmermann, 2007). The number of inoculated insects in the host population may have increased mortality given that the highest mortality was achieved at a high vector ratio of 50 % (Kocaçevik *et al.*, 2016). Moreover, the sex and aggregation pheromones produced respectively by female and male BRB (Uzakah *et al.*, 2015) and attraction to banana corm pieces (in containers for our study) through olfactory means volatiles had surely facilitated movement and mixture of marked (inoculated) males and females with non-inoculated males and females under the corm which served as food. BRB behavior could have enhanced the auto-dissemination of the pathogen in the host population. Earlier studies also showed that *C. sordidus* (Lopes *et al.*, 2011) as well as ants (Pereira and Stimac, 1992) and f *Microcerotermes diversus* Silvestri (Isoptera: Termitidae) (Cheraghi et *al.*, 2012) are an effective carrier of EPFs. Though epizootic, infection of non-inoculated insects is not predictable, the output of this study can be manipulated to control and limit BRB outbreaks in the field by reduction of the host population (Vega *et al.*, 2012). Attract-kill strategy that combines kairomone or pheromone with EPFs can enhance the transmission potential of the fungus via living insect that comes in contact with infected host cadavers and transferring the inoculum (Nankinga *et al.*, 1999; Uzakah *et al.*, 2015). Studies have shown that a biopesticide delivery system in the field can be implemented for BRB control (Nankinga and Moore, 2000; Godonou *et al.*, 2000; Tinzaara *et al.*, 2007*;* Godonou *et al.*, 2000; Tinzaara *et al.*, 2007; Tinzaara *et al.*, 2015; Moreira *et al.*, 2017; González *et al.*, 2017; Opisa *et al.*, 2019). Such information can help in developing and implementing an effective delivery system of an eventual biopesticide for BRB control in Cameroon and the rest of Central Africa.

Conidia production on BRB cadavers was fungus isolate-dependent with significantly higher conidiation for BIITC6.2.2 compared with all other isolates. High conidia yield is important for explosive epizootic which can lead to a disease outbreak in favourable conditions and consequently lead to reductions in the host population (Myers *et al.*, 1995). The sporulation potential of BIITC6.2.2 is of great importance for commercialization and the potential for field epizootics. The difference in conidial yield between EPF isolates and species were also reported by Bayissi *et al.*, (2016) and Mweke *et al.* (2018) on aphid cadavers.

Cameroonian isolates showed wide pH tolerance and optimum ranges. Germination, mycelia growth, and conidiogenesis allowed at acidic to alkaline medium. The tolerance and optimum range for germination were 3 - 13.5 and 3 - 13.5 respectively while it was 3 - 13 and 4 -

12 for mycelia growth and 3-13 and 3-13 for conidia production. However, the tolerance and optimum ranges depended on fungal isolates and biological traits studied. pH 2 and 14 were very toxic for germination while pH 2, 3, 13.5, and 14 were highly toxic for mycelia growth and conidia production. These results indicate that mycelia growth and conidia production are the most sensitive saprophyte phase in terms of pH. These two phases are determinant for disease spread in the host population and the industrial production of fungi. It is surely because mycelia growth is a critical saprophyte phase where fungus hyphal tube penetrates the subtract for nutrient uptake. Many reports showed that *B. bassiana* can tolerate 5 - 6 (Sanzhimitupova, 1980), 6 - 8.5 (Galani, 1988), 10 to above (Shimazu and Sato, 1996), 4 – 14 (Padmavathi *et al.*, 2003), and 2-12 (Otgonjargal *et al.*, 2015). Earlier reports on pH tolerance range for *M. anisopliae* was 4 - 7.8 (Rath, 1992), 5.5 - 8.5 (Kotwal *et al.*, 2012), 2.21 - 10.5 (Hallsworth and Magan, 1996), 4 -9 (Teja and Rahman, 2016). The tolerance and optimum range found in the present study for *B. bassiana* and *M. anisopliae* isolates has not been reported in earlier studies. The genetic diversity and ability of EPF to modify the pH of a medium according to its desire can explain the large range of pH tolerance and optimum ranges in our study (St Leger *et al.*, 1998; Padmavathi *et al.*, 2003; Raya-diaz *et al.*, 2017). The tolerance range for fungus growth depends on the pH level studied. However, Cameroonian isolates fill Industrial requirements related to high-quality inoculum able to grow well at pH media below neutral which are desirable to inhibit contaminant growth (Barlett *et al.*, 1988).

Our study also revealed that an increase of the incubation period increased tolerance and optimum ranges for the germination of fungi. The pH 13 and 13.5 did not prohibit but delayed germination. The pH 3 was toxic for mycelia growth but not for conidia production. The occurrence of conidia production at pH 3 and 13 may have been micro-cycle conidiation since mycelia growth was inhibited (Jung *et al.*, 2014). Lui *et al* (2015) revealed the presence of twenty-eight metabolites in *B. bassiana* mycelia, the decrease or increase of these metabolites may have protected spores against the harmful effects of acidic pH. Similar results were obtained by Padmavathi *et al* (2003) who reported that pH 3 was toxic to mycelia growth of 29 *B. bassiana* strains but did not prohibit germination.

Our results from the photoperiod effect on fungal growth showed that white light periodicity affects fungi development with mycelial growth as the most sensitive saprophytic phase among biological traits studied. The response of fungi to photoperiod regimes also depended

a lot on isolates. Beauveria BIITAC6.2.2 developed well in continuous dark and 12:12LD while Metarhizium MIITAC11.3.4 expression was unstable, good germination under continuous light and highest mycelia growth and conidia production at 12:12LD. Various studies have been reported the effect of light regimes on diverse fungi genera but there is very few reports on Beauveria and Metarhizium (Alves *et al.*, 1984; Onofre *et al.*, 2001; Zhang *et al.*, 2009; Pittarate *et al.*, 2016). Our findings are contrary to previous reports except that continuous light was the pre-requite for the highest germination of Cameroonian *M. anisopliae* isolate, MIITAC11.3.4 as reported by Rangel *et al* (2011). This difference can be related either to fungal adaptability to light exposure or interspecies variation.

The color gradient of the fungi BIITAC6.2.2 and MIITAC11.3.4 increased with the photophase extension. *B.bassiana* and *M. anisopliae* when from a white cream to yellow and grey-green to olive green respectively at dark to continuous light exposure. The appearance of the yellow coloration in the isolate of *B. bassiana* showed that this isolate can absorb the light which induced the synthesis of carotenoid, as is the case in certain fungi characterized by the yellow coloration (Avalos and Limon, 2015). The gradual increase of yellowish staining could indicate carotenoid content and consequently survival (Moline *et al.,* 2009). These carotenoids play a prominent role in photoprotection against oxidative stress and UV solar radiation (Braga *et al.*, 2015). These carotenoids mostly absorb visible light where they act as quenchers of photosensitization products and as inhibitors of free-radical reactions (Gao and Garcia-pichel, 2011). Additionally, the melanin pigment located in the cell wall complexed with proteins and other compounds, synthesized during conidiation may be responsible for the olive green coloration of Metarhizium (Braga *et al.*, 2015). However, the pigmentation change induced by a period of light exposure may be due to the disruption of the MrPks1 and Mlca1 enzymes involved in the conidial pigmentation of Metarhizium (Chen *et al.*, 2016). The disruption of enzyme Mlca1 can also affect virulence and tolerance of conidia to UV-radiation and heat shock (Braga *et al.*, 2015). The deleterious effect of solar radiation and high temperature to whom fungi are exposed during their life cycle has led to the development of defence mechanisms involving pigments, enzymes, cell metabolites, and DNA repair systems (Braga *et al.*, 2015).

UVB light significantly affected Cameroonian fungal isolates growth compare to the unexposed situation, with high UVB tolerance. Beauveria isolate remained active during all exposure times while Metarhizium isolate did not form colonies at 45 min exposure under UVB

light even when the incubation time was extended by 6 days. However, mycelia growth and conidia production was not prohibited at 45 min exposure. We also observed that extension of incubation time after exposure to UV-light increased colony formation, especially for *B. bassiana* isolate. The results suggest that UVB light can delay colony formation but not prohibit mycelia growth and conidia production. The delay of germination by UV light was also obtained by Zimmermann (1982) while testing EPF germination under UV light. Numerous reports have shown action of UV at different radiation lengths against EPF (Ottati-de-Lima *et al*., 2014; Fernandes *et al*., 2015; Rodrigues *et al.,* 2016). The similarity of earlier reports with our results is that UV light can delay fungal growth. The difference in isolates UV B tolerance with previous reports could be related to fungi specificity, the temperature of UV simulator, the color of UV light, and radiation region of wavelength used in studies. It is evident that additive acting as sun blocker or sunscreens will be needed for biopesticide formulation process to protect active ingredient against UV irradiance as referred by Zhang *et al*. (2016).

Exposure time for 30 min under UV light stimulated mycelia growth and conidia production for BIITAC6.2.2 and only mycelia growth for MIITAC11.3.4. From studies on UV action to EPF, Zhang *et al* (2009) also observe stimulation of conidia production when *B. bassiana* mycelia was exposed to blue light for 3 min. However the results of Zhang *et al*. (2009) also revealed that purple light (280 - 400 nm wavelength) had relative negative effect on conidia production. According to Sahab *et al* (2014), 30 min exposure of *B. bassiana* strains from Montpellier to UV light (253.7 nm wavelength) resulted a mutant with positively high activity against insects. It have been demonstrated that UV light color affects conidia reproductions but not radial growth with significant low conidia reproduction when mycelia is expose to purple light (Pittarate *et al*., 2016). Our findings support the fact that UV light can simulate conidia production as proven by Zhang *et al* (2009), but our report however shows for the first time a positive effect of purple color UV light on mycelia growth and conidia production compare to results of Pittarate *et al.,* (2016) who presented purple UV light as detrimental to conidia reproduction.

Fungi development was effective at low to high humidity. The effect of RH depended on fungal isolate with high growth and conidia production of *M. anisopliae* MIITAC11.3.4 and *B. bassiana* BIITAC6.2.2 at moderate relative humidity. However, *B. bassiana* and *M. anisopliae* exhibited the highest colony formation at moderate to high humidity (75 – 99 %) and low RH (30

%) respectively. It have been reported that moderate to high humidity are required for fungal germination, optimize conidia production on insect cadavers, industrial production of conidia on solid substrate, storage of conidia and survival of conidia on the release environment (Athurs and Thomas, 2001; Arzumanov *et al*., 2005; Zimmerman., 2007 ; Sandhu *et al*., 2012; Barra-Bucarel *et al*., 2016). Our findings are closer to results obtained by Sosa-Gomez and Alves (2000) who showed conidia production of *B. bassiana* on insect *Diatraea saccharalis* was high at 75 % RH. Our findings support the fact it is important to study the effect of environment factor when dealing with a new isolate to determine formulating design, simulate response of fungi at insect target habitat (Mishra *et al.,* 2015). Given that some insect like *C. sordidus* though prefers high humidity environment can survive in dry environment, our strain are likely for such critical insect as well other insects which need to be assessed.

The present study showed that quality of conida mass-produced varied depending on parameters studied. The insecticidal potential was similar for both fungal and low than 70 %. The virulence of isolate study here was previously reported high, > 93 and 84 % mortality respectively for BIITAC6.2.2 and MIITAC11.3.4 against the same insect (Membang *et al.,* 2020). The methodology used by Membang *et al.* (2020), 24 h starvation of insect after inoculation with fungi (which was not used in this study), would have weakened the immune system of the host. The insect population origin and length of incubation time may explain difference in virulence with Membang *et al*. (2020) as suggested by Lopes *et al*., (2011).

The *B. bassiana* BIITAC6.2.2 had high conidia viability, high conidia amount and absence of contaminant compare to Metarhizium MIITAC11.3.4 isolate contaminated with *Rhizopus* sp.

Similarly to our findings Moslim *et al.* (2005), demonstrated that *Rhizopus* spp. can contaminate *M. anisopliae* mass-produced dry conidia. Competitivity potential of a strain is also an important factor to be considered given that contamination could occur at any of several stages of the production process, especially during the extraction process where extracted conidia remain exposed in the laboratory, it needs to be below 0.002 % (Jenkins *et al.,* 1998). However, the contamination problem can be solve using proper microbial sterilization and hygienic procedure, though it may increase production cost (Cherry *et al*., 1999; Moslim *et al*., 2005).

Conidia viability obtained with this same isolate here was high for *B. bassiana* BIITAC6.2.2 but low for M. anisopliae MIITAC11.3.4 compare to report of Membang *et al.* (2020) who showed that when newly isolated from soil, conidia viability of BIITAC6.2.2 and

MIITAC11.3.4 are respectively < 80 % and > 99 %. similar report was obtained by Dangar et *al.* (1991), Latifian *et al* (2013) and Ibrahim *et al* (2015) who showed that conidia viability of *B. bassiana* and *M. anisopliae* from mass-produced dry conidia was higher conidia viability than obtained from artificial culture media. Their statement was confirmed for *B. bassiana* but not for *M. anisopliae* probably due to presence of a contaminant which would have affect conidia viability of *M. anisopliae*. Variation of production system (type of substrate, incubation conditions and harvesting method), number of sub-culturing prior mass-production, genetic and intraspecific variability could justify difference of conidia yield and conidia viability herewith from earlier findings (Jenkins et *al.*, 1998; Cherry *et al.*, 1999; Kassa *et al.*, 2008; Moslim *et al.*, 2005; Ansari and Butt, 2011; Ibrahim *et al.*, 2015).

Most inactive ingredients used in this study were available in markets around Nkolbisson except carrot powder, banana flour, wood ashes and mucuna. The rarity of the four mentioned ingredients could be due to lack of knowledge on their various used. Carrot flour could be used for insect rearing in biological control of fruit fly parasitoids (Nanga *et al.*, 2019), banana flour for preparation of bread, cake and vin; wood ashes for control of insects and stabilization of pH of high acidic soil; and mucuna to improve soil fertility and ruminant feeding in the Nord region of Cameroon and as cover crop in the West and Central Africa (Hauser and Nolte, 2000; Tedonkeng *et al.*, 2010). Ashes also contain nutrients that fungi can mobilize for their growth (Mahmood *et al.*, 2003).

Most additives were also cheap except carrot, baobab and milk powder. Charcoal and kaolin were the cheapest ingredients. The low price of these ingredients expressed high availability of wood in tropical humid forest and high availability of Cameroon kaolin described as potential industrial kaolin with high silica contain and plasticity index (Nkoumbou *et al.*, 2009; Mefire *et al.*, 2016), which needed investigation on used according to Nkoumbou *et al* (2009). The wide use charcoal and kaolin as carriers and UV protectants could be explained by effectiveness and cost (Inglis *et al.*, 1995; Sudneendrakumar *et al.*, 2006). Similarly, Behle *et al.* (2009); Cohen *et al.* (2009) and Posadas et *al.* (2012) also highlighted that the price of the formulated product depends on the availability and cost of additives used by the formulator.

The results showed two different scenarios, some ingredients reduced conidia viability while others stabilized conidia viability with time. The high viability of conidia was observed when mixed with banana flour, cassava starch, maize flour as well as kaolin and charcoal. The

composition of ingredients that could be different as well as humidity can explain the difference in response. Several studies exist on the effect of adjuvants or carriers on the efficacy of entomopathogenic fungi for the control of specific insect pests, but few studies are available on shelf-life for formulated conidia in powders (Wakil *et al.*, 2015; Bukhari *et al.*, 2011). Our results corroborate in one part with findings of Moore *et al.* (2005) who showed that the viability of entomopathogenic fungi reduces with storage time. Simkova (2009) also revealed that nutritive carriers can better promote high shelf life compare to unformulated conidia and inert carriers. However, kaolin and charcoal contained silica which plays an important role of desiccant industrially manufactured (Marray, 2000 ; Mefire *et al.*, 2016). The presence of silica in kaolin, could have contributed to maintaining low moisture in pre-formulation, favoring the survival of conidia. Other studies suggested that charcoal can play the role of desiccant as silica gel (Wakil *et al.*, 2010).

The water holding capacity depended on additives with high water holding capacity for gari, carrot, baobab and mucuna powders and low WHC for cassava starch, wood ash and kaolin. The difference in water absorption could be related to the degradability potential of additives. According to Altschul and Wilcke (1985), high WHC results in fast degradation which can favor growth of contaminants and consequently competition between saprophytic organism and the active ingredient. Therefore additives with low WHC are desirable for formulation development. A similar approach have been developed for the production of fungi on nutrient-impregnated clay micro granules which are sometimes applied directly in the field (Riba, 1984, 1985, Godonou *et al.*, 2000). Kaolin have shown synergic effect on fungus *B. bassiana* (Storm *et al.*, 2016)

The field application of a biopesticide requires an adequate system to spread and deposit the microbial agents. The choice of correct equipment is therefore essential to ensure that the biopesticide is applied effectively at the correct rate and in the target surface. It depends, among other things, on the size of the particles entering the compositions of the final products. Most equipment for biopesticide application has nozzle droplet size between 10 - 200 µm (Gan-Mor *et al.*, 2014). Development of an easy applicable biopesticide most therefore take in account the delivery system for the application of the field. Thus, it is important to determine the size of the particle and flow properties which are parameters that can help to predict the distribution and to choose the ingredient that may favor good distribution of the active ingredient.

Our result revealed that six of tested inactive ingredient wood ashes, cassava starch, banana flour, mucuna powder, maize flour and kaolin clay retained the lesser quantity of ingredient on the sieve 100µm, meaning that, they have very fine particles that can easily spread with pesticide application equipment which have nozzle droplet size between 10 – 200 µm (Gan-Mor et al. (2014). Also, rice flour, gari, wood ashes, charcoal and milk powder showed fair to excellent flowability potential. The difference in flowability between ingredients could be related to particle morphology and moisture content as reported by Berretta et al (2014) and Crouter and Briens, 2014 while using pharmaceutical powders.

The pH level of ingredients varied depending on ingredients with most ingredients acidic (pH level 3.16 – 6.72) except wood ashes and charcoal which were basic with respectively 11.04 and 9.71 pH value. The contains in H30+ and OH- determine the pH level of ingredients. Variation of pH means that the contain of the ions mentioned above varied a lot between ingredients. Membang et al (submitted) reported that the pH range 3 -13 was favourable for Beauveria and Metarhizium growth. The range of ingredients studied is also contained in pH range of fungi activity. The pH of ingredients kaolin, carrot powder, banana flour, mucuna powder, cassava starch, maize flour, rice flour and milk powder were at the recommended interval for biopesticide registration as settle by the environment protection agency (EPA), pH between 4.41 and 7.4. Additionally, charcoal and wood ashes can be used as stabilizers. Wood ashes induce pH increase have been used to sprinkle soil surface to obtain acceptable pH for high acid soil and non-acid-loving plants (Anonymous, 2007). However, wood ashes should not be contaminated by metal otherwise it will have a negative effect on plant growth due to element accumulation in plant and soil quality (Jones and Quilliam, 2014).

Our founding's revealed that none of the ingredients had less than 5 % relative humidity (RH), but banana flour, cassava starch, and rice flour showed closer values. However, they need to be dry longer. This difference can be attributed to the drying process or water holding capacity. This parameter is very important given that it affect biopesticide shelf life.

Results on the effect of UV light and sunlight on conidia viability, when mixed with ingredients, showed that sunlight significantly affected conidial viability compare to UVB light. It is well known that sunlight contains apart from UVB, UVA, UVC and infrared which may have also contributed to inactivation of some conidia. Our findings corroborate with those of Braga et al (2001d) who showed that sunlight can have deleterious effect on insect pathogenic fungi.

Greater protection to both UV-B and sunlight was offered by charcoal, banana flour, kaolin clay and cassava starch. The difference in particle size distribution, pigmentation and biochemical composition could explain these results (Du *et al*., 2010; Hoangmi *et al*., 2010). Similarly, Shapiro *et al.* (1995) cited UV irradiance among factors influencing the environmental degradation of biopesticides. Several reports highlighted clay, starch and carbon (charcoal) as effective UV protectants for their easy degradability, good protection in the field and cost-effectivity (Sudneendrakumar et *al.*, 2006, Behle *et al.*, 2009; Cohen *et al*., 2009, Posadas *et al*., 2012).

Storage temperature and duration influenced shelf life as reported by earlier reports (; Kassa *et al*., 2004; Sy *et al*., 2016; Przyklenk *et al*., 2017; Gola *et al*., 2019) Though co-formulations had moisture content above 5 %, they keep conidia viability above 90 % at -50, -20 and 4 °C after more than 60 weeks while faster decrease of conidia viability was observed at 25 °C as reported by Przyklenk *et al* (2017). The rapid decreased of conidia viability at 25 °C revealed that this temperature was not adequate for medium to long term storage of BIITAC6.2.2 formulated and unformulated. Our findings at 25 °C are closer to Mbarga *et al.* (2014) who showed that *Trichoderma* can reduce viability from 90.6 to 20.9 % after 35-week storage at room temperature. The slight difference is due to type of formulation, oil instead of powder in our case, carriers and biological agent genera. Gola *et al* (2019) instead showed that myco-granule formulated with Beauveria can prolonged shelf life compared to myco-tablet and myco-capsule, meaning that type of formulation can also affect shelf life. The presence of nutritive ingredients in all formulations could explain why conidia viability did not differ between formulations. Similarly, Šimková *et al* (2009) reported that nutritive carriers were the most suitable for the storage of *B. bassiana* conidia, especially when kept at low temperatures. They also demonstrated that temperature 4 °C and -20 °C was suitable for the stability of *B. bassiana* conidia. Lacey (2012) also demonstrated as in our study that short to medium term storage at – 20 °C or – 80 °C had no adverse impact occurred on DNA quality and DNA (relatively stable within cells) of entomopathogenic fungi. The ability of our formulations to keep more than 90% viability after 72 weeks (1.5 years) is desirable for biopesticide commercialization, time range acceptable (12 – 18 months) reported for BotaniGard and GreenMuscle (Couch and Ignoffo; Milner, 2000; Kim *et al*., 2010).

The formulations tested in this study showed high insecticidal potential compare to co-formulants, revealing that co-formulant did not have an effective insecticidal effect on the adult banana weevil. The difference of response observed between formulated and unformulated conidia against adult weevil can be due to the difference in conidia dose of each formulation related to dryness and density of carriers. It was expected that the non-formulated spores would be more infectious because they were more abundant (high dose as it was essentially conidia) and could attach the cuticle all over the place. It turns out that the formulations were subject to rapid and high infection. The latter would have certainly favored the conidia's adhesion and synergistic effect on the cuticle of the insect reported by Storm *et al* (2016). Our results here confirmed the findings of Mahot *et al* (2019), Nuzibizi (2018) and Membang *et al* (2020) who reported high virulent of the isolate Beauveria BIITAC6.2.2 against cocoa mirid, okra beetle and BRB.

Formulations did not affect plant growth negatively meaning that these formulations were non-phytotoxic as reported by Davi and Hari (2009) while testing the effect of *B. bassiana* concentrate against *Helicoverpa armigera*. Zimmerman (2007) also highlighted that *B. bassiana* is not phytotoxic. The difference observed on growth parameters between infested and un-infested plants indicates the importance of banana weevil on cultivar used in our study. However, Akello *et al* (2008) revealed that *C. sordidus* affect mostly pseudo-stem diameter which was not confirmed in this study. The protective role, as well as multiples roles such as endophytes, mycorrhize and growth promoters, may have been involved to stabilize plant growth (Dara and Dara, 2015a; Jaber and Enkerli, 2016).

Our results revealed that reproduction of weevil varied with formulations with kaolin, cassava-based formulations for post-infestation method and maize, banana and cassava-based formulations (for pre-infestation) reducing considerably reproductions. The important number of immature weevil observed in some treatments could mean that oviposition took place earlier since insects used for infestation were sex for days before the infestation of plants in the greenhouse. Simmonds and Simmonds (1953) reported that a single weevil larva can cause catastrophic damage by giving a young sucker no chance of recovery (Uzakah *et al*., 2015). Akello (2007) found a similar results while testing the endophytic potential *B. bassiana* G41. However, in our study the total number of immature weevil was low when treatments were applied before infestation, showing that eggs probably led were mycosis or some larvae which emerged from eggs died.

B. bassiana BIITAC6.2.2 drastically reduced the incidence and damage (severity) caused by the immature stages by 24 - 95 % (central cylinder) and 23 – 60 % (cortex), showing the protective potential of microbial formulations tested in this study. Twesigye *et al* (2018) obtained peripheral damage of 4.8 – 50.4 % when assessing the damage level variation caused by banana weevils collected from different banana growing regions of Uganda. The report of Nankinga (1999) is in agreement with our findings. She demonstrated that application with fungi was more effective than without fungi. The low incidence and damage of some treatments compare to others may be related to the facility of a formulation to be picked by insects (Godonou, 2000), which can result in high and fast mortality and consequently low reproduction and low damage.

The insecticidal potential of microbial formulations in the greenhouse indicated the effectiveness of *B. bassiana* BIITAC6.2.2 against banana weevil using both application methods. The high performance of formulations studied could be related to the high virulence of *B. bassiana* BIITAC6.2.2, causing up 92 % mortality against adult weevil in laboratory conditions (Membang *et al.*, 2020). However, the persistence of conidia in the soil differed with formulations and decreased with time particularly for T2, T3 and T4 compared to T1. These formulations were based on nutrient ingredients, meaning that degradation related to mycoparasite could have occurred during the trial and affect survival or activity of conidia as observed on maize substrate mixed with clay. (Magara *et al.,* 2003) The reduction of inoculum on soil samples collected each month for evaluation of persistence can also justified a decreased of mortality. According to Nankinga *et al* (1994), *B. bassiana* conidia could last in the soil under laboratory conditions for at least two years. In our study, we did not evaluate conidia viability for up to 2 years as studied by Nankinga *et al* (1994).

Among the growth parameters evaluated in our study, stem girth was the only one that varied with treatments, treatment T1 showed the lowest stem girth. The dimension of plant girth have been reported to be influenced by infestation of banana weevil *C. sordidus* (Akollo *et al*., 2008, Armendariz *et al*., 2016.). According to Rukazambuga *et al* (1998), moderate and heavy attack of banana weevil can reduce among other parameters plant girth. We observed that during plant growth, the weevil population was high on treatment T1 and the damage level was moderate meaning that the more population is high the more damage can be high and consequently destruction of tissue will be severe and serve uptake affected. Non-phytotoxicity was also confirmed as reported by Devi and Hari (2009).

The dynamics of the population have been evaluated in our study during the four seasons meet in Yaounde. There was a variation of the population, however, the environmental factors had no direct effect on the fluctuation of the population in our experimental site. A fluctuation of the population is usually observed over time (Fancelli *et al.*, 2013; Sintim *et al.*, 2016). Some researchers in Latin America have attributed this fluctuation to environmental factors (rainfall, temperature and relative humidity), while in Africa researchers have shown the opposite. Okolle et *al* (2009) in Cameroon showed on studies in two agroecological zones (littoral and south-west) that the fluctuation of the banana weevil population over time depends on the agroecological zones. Little information exists on the dynamic of banana weevil populations in the central Cameroon region. The difference in banana weevil population dynamics could be attributed to the trapping system and level of infestation in fields studied. This investigation also showed no correlation between captured weevil and rainfall as obtained by Tinzaara et *al* (2005b); Rhino et *al* (2010); Sintim *et al* (2016). Contrary from our findings many researchers found relationship between catches weevil and temperature and or relative humidity (Tinzaara *et al* (2005b); Rhino *et al.*, 2010; Duyck *et al.*, 2012) .The results may also depend on trapping system.

We also observed that when applied alone, formulations showed low infectivity in the field specially at 2nd and third application. However, cadavers showing fungi out growth were observed in the field for treatments T1 and T2 during the evaluation period.

The high population of *C. sordidus* for biopesticide application treatments compares control could be due invasion of weevil from rotten stump found in the fields around our experimental site. According to experimental design, weevils from destroyed fields could have easily invade the corners (where treatments T1 and T2 were found) than the middle where control treatment were found since the source of infestation was around the field. The lack of attractant after application of formulation could have influence results since attractant, pseudo-stem were placed on week after application. Nankinga *et al* (1999); Magara *et al* (2003) reported that *B. bassiana* based formulations were effective for the management of banana weevil when applied in an interval of 90 days while Moreira *et al* (2017) by applying wettable powder Boveril based on Beauveria under pseudostem trap at interval of 14 days in the field, against banana weevil *C. sordidus* achieved population reduction above 75 %. Similarly, Gonzalez et *al* (2018) applied liquid formulation of Beauveria and Metarhizium every 15 days and they obtained up to 87.7 % mycosis of adult weevil population. Fancelli et *al* (2013) also found low population with biopesticide compare to control.

A low reduction in our study could have resulted from long interval of application (6 months and 10 months) and application methodology. Many researchers have proven that use of the attract kill method is more efficient and can increase the performance of a biopesticide (Tinzaara *et al.*, 2007; Lopes *et al.*, 2014; Tinzaara *et al.*, 2015; Armendariz *et al.*, 2016).

The study showed that damage and bunch weight were not significantly different between treatments and control. As far as studies of banana weevil management using biological tools are concerned, the major researches only evaluate the population of weevils (alive or dead), less studies extended research by assessing damage (Nankinga *et al.*, 1999; Godonou *et al.*, 2000;; Tinzaara *et al.*, 2007; Fancelli *et al.*, 2013; Tinzaara *et al.*,2015; Armendariz *et al.*, 2016; Gonzalez *et al.*, 2018; Moreira et *al.*, 2017).

In field trials conducted by Sintim *et al* (2016), they also found low damage of up to 14 % at the first cycle. The difference on damage may be due to difference of genotypes that may be susceptible or moderate resistant (Oliviera *et al.*, 2017). Nankinga *et al* (1999) also highlighted that damage was not significantly different between plots treat maize-based biopesticide and control at the first cycle while Armendariz et *al* (2016) showed a difference on bunch weight between pheromone plot and fungi plot, with high bunch weight for pheromone plot. Young banana plants are most at risk because tunneling by the banana weevil can be fatal at this stage. The high attractiveness of the pheromone trap reported by Tinzaara *et al.* (2005); Rhino *et al.* (2010); Duyck et *al.* (2012); Figueroa-catro et *al.* (2017) could explain the difference between our results and those of Armendariz et *al* (2016).

Results of conidia persistence showed that Beauveria conidia can persist in soil under field conditions for at least 8 months. The presence of infected *C. sordidus* after 10 months from 3rd application shows that inoculum was still present in treatment T1 (Personal observation). Treatment T1 represents plots treated with the clay-based formulation. Additionally, high mycosis rate was achieved from soil of treatment T1. The clay high carrying capacity, its protectant function against biodeterioration and its ability to increase stability and longevity could explain the good persistence on treatment T1 (Davet,1983; Fargues et *al.*, 1983; Keller and Zimmermann,1987; Vanninen *et al.*, 2000). Nankinga *et al* (1999) demonstrated that Beauveria conidia can persist in the soil for at least 90 days. Other authors proved that Beauveria conidia can survive for more than a year (Wojciechowska *et al.*, 1977; Vanninen *et al.*, 2000). The persistence depends on type of formulation, technic of implementation, soil type and environmental factors (Vanninen *et al.*,

2000; Inglis, 2000; Sweirgiel *et al.*, 2016). The formulation applied on treatment T2 was based on nutrient carriers, consequently could have favors high microbiological activity and the presence of mycoparasites of entomopathogenic fungi *B. bassiana* (Fargues *et al.*, 1983). The quantity of inoculum can decrease or increase with time (Vanninen *et al.*, 2000; Sweirgiel *et al.*, 2016) related to rainfall and abundance of insect host.

The results showed that pheromone associated to pseudo-stem trap caught 17 times more weevils than pseudo-stem trap alone. The high population of weevil caught on trap made of cosmolure combined with pseudo-stem reveals a possible synergetic effect. The synthetic pheromone (release 3mg/day for 90 days) and plant keromone released by cosmolure and pseudo-stem respectively are known to be both attractant of weevil. Our findings are not far from those of Tinzaara *et al* (2000) who caught 18 times more banana weevil *C. sordidus* with pitfall trap associate to cosmolure, compare to pseudo-stem trap. However, the fact that cosmolure was attached on pseudo-stem and expose to rain may have affect the quantity of release odor (Saïd *et al.*, 2011).

Our findings revealed that formulations caused significant high infectivity with up to 83.33 and 78 % mortality and mycosis rate. The high performance of formulation could be attributed to the presence of attractant (trap of pheromone combined to pseudostem) which could have favor contact of weevil with formulation. Application of biopesticide alone or in combination with kairomone, pheromone and both have been tested with different technics (Nankinga *et al.*, 1999; Godonou *et al.*, 2000;; Tinzaara *et al.*, 2007; Fancelli *et al.*, 2013; Tinzaara *et al.*, 2015; Armendariz *et al.*, 2016; Gonzalez *et al.*, 2018; Moreira *et al.*, 2017; Queiroz *et al.*, 2017). Moreira *et al* (2017) while applying wettable powder Boveril based on Beauveria under pseudostem trap interval of 14 days in the field, against banana weevil *C. sordidus* achieved population reduction above 75 %. Similarly, Gonzalez et *al* (2018) applied liquid formulation of Beauveria and Metarhizium every 15 days and they obtained up to 87.7 % mycosis of adult weevil population. Fancelli *et al* (2013) also found low population with biopesticide compare to control. Our findings are closer to those of Moreira et *al* (2017) and Gonzalez et *al* (2018) the slight difference could be attributed to environmental conditions, season of formulation application, type of formulation and distance of application point evaluated. Tinzaara et *al* (2015) for example check for insect up to 9 m from application point while in our study we evaluated mortality and mycosis at 50 cm only.

The biopesticides also caused dead of other banana pests though they are minor pests (Okolle *et al.*, 2009). They surely contacted the inoculum either by walking on the formulation or by horizontal transmission. This is a great advantage for the dissemination of inoculum since *Metamacius* can fly (Lopes *et al.*, 2011).

CONCLUSIONS, PERSPECTIVES, AND RECOMMENDATIONS

Conclusions

Biopesticides are important IPM tools and are safe alternatives to pesticides that increase every year in the market of crop protection products. *Beauveria bassiana* and *M. anisopliae*, two entomopathogenic fungi mainly used as mycoinsecticides are known to be infectious to various agricultural pests including *C. sordidus*. Discovering new and indigenous isolates with high virulence and infectivity to all life stages of the target pest can give added value by reducing mycoinsecticide's production cost and property right. Many studies have been performed worldwide to develop microbial biopesticide product against various pests but very few products are base in African indigenous isolates. The low number of products can be attributed to complexity of formulation technology. Therefore this study was initiated to select an effective active ingredient and developed performant microbial formulation against *C. sordidus*. Following conclusions can be done after investigations from laboratory to field bioassays.

The six indigenous Cameroonian isolates were found promising against the BRB. Apart from being pathogenic and virulent to multiple stages, these isolates transmitted disease from one life stage to the next. These output are an important attribute in disease epidemiology that increases efficacy against host populations and offers an unlimited opportunity, to Cameroon and Central Africa in general, to develop biopesticides against various pests and in various cropping systems using local isolates. The virulence of isolates studied could result from insecticidal potential only since endophytic properties were not confirmed with the methodology performed.

Temperature significantly affected all biological traits (namely germination, mycelia growth and conidia production) examined in this study, with 37 °C being most detrimental temperature. Thermo-sensitivity was most pronounced for conidia production on artificial media and fungus isolate-dependent. Cameroonian entomopathogenic tolerated wide range temperature 13 – 33 °C. The optimum temperature ranges for fungi germination and sporulation obtained in this study is both close to thermal range for banana crop production, banana root borer development and activity, and favourable temperature for fungi infectivity (for several hosts including *C. sordidus*) and disease occurrence. These isolates can be classified as mesophilic. Beauveria isolates, BIITAC6.2.2 and BIITAC10.3.3 Metarhizium MIITAC6.2.2 and MIITAC11.3.4 showed a good profile in term of tolerance. The Horizontal transmission occurred with the four thermotolerant isolates (mentioned above), but the mortality of non-inoculated insect was highest for the isolate BIITAC6.2.2 followed by MIITAC11.3.4. Taken together, the wide

range of thermal tolerance, pathogenicity and capacity of transmission within the host populations support the selection BIITA 6.2.2 and MIITAC11.3.4 as a suitable candidate for the development of a mycoinsecticide for use in IPM of banana root borer in Cameroon and likely in regions of Central Africa with similar conditions.

Environmental factors affected significantly the development of Cameroon entomopathogenic fungi isolates on artificial media. The fungi strain MIITAC11.3.4 and BIITAC6.2.2 have a wide range of tolerance for growth, acid, near neutral and alkaline conditions. For the optimum growth they need to be cultured at pH range 4 – 11 and 4 - 12 respectively for MIITAC11.3.4 and BIITAC6.2.2. Therefore, they are recommendable for mass production. BIITAC6.2.2 is less exigent to light and can be suitable for control of insect of all habitat while MIITAC11.3.4 give optimum development in total dark and 12light:12 dark. The two isolates tested in this study were found tolerant to UVB-light. Though UVB-light delaying conidia survival, it stimulated conidia production. The fungi isolate MIITAC11.3.4 is more stable while BIITAC6.2.2 can produce a mutant after 30 min exposure to UVB-light. Infection potential of this mutant needs to be tested. MIITAC11.3.4 and BIITAC6.2.2 could also grow at low to high humidity environment but moderate humidity environment was preferable for their best expression. However, the insecticidal potential needs to be assessed in a low to high humidity environment.

Based on the conducted research, the importance of quality control of the final product (spore powder), have been proven, for decision making to choose a cost-effective active ingredient for biopesticide formulation and commercialization. Mass-produced conidia of Beauveria BIITAC6.2.2 showed high quality compared to MIITAC11.3.4, but the contamination problem which occurred with mass-produced conidia of MIITAC11.3.4 can be solve using more hygienic and proper methods. Kaolin clay, cassava starch, maize flour and banana flour were found to be best carriers of BIITAC6.2.2 dry powder while charcoal and wood ashes can be used as stabilizer. Our findings also demonstrated that kaolin clay, cassava starch, banana flour and charcoal are effective UV protectants.

Potential of the four formulations developed varied from one condition to another. Laboratory conditions, formulations conserved good stability at 4 °C, -20 °C and -50 °C for up to 72 weeks. The four formulations enhanced insecticidal potential of active ingredient, causing up to 100% mortality in 9 days' post inoculations of adult banana weevils.

Results of greenhouse trial indicated that *B. bassiana* BIITAC6.2.2 formulations can be used both for post-infestation and prevention of infestation. Microbial formulations developed had non-phytotoxic effect on plantain cultivar treated. Formulations applied with pre-infestation method reduced banana weevil reproduction compare to post-infestation method and control. The number of immature *C. sordidus* was low when plants were treated with cassava-based formulation (T4) and kaolin-based biopesticide formulation (T1) using post-infestation method. The protective potential of the biopesticides tested in this study was also shown with low relative incidence and reduction of damages due to *C. sordidus* in the cortex and center cylinder where formulations were applied compare to control. Additionally, microbial formulations studied killed more than 84 % artificial infested adult weevil, *C. sordidus*.

Non-phytotoxicity of formulation tested to plantain cultivar used was confirmed from field where biopesticide formulations were applied alone. mycoinsecticide treatment did not show clear positive effect on damage, insecticidal potential and yield for the first crop cycle. However, two non-target insect species showed signs of mycosis apart from *C. sordidus* (*Pollytus mellerborgi* and unidentified insect). The inoculum in both formulations applied in the field persisted for more than 8 months after application with kaolin clay-based formulation persisting better than cassava starch based formulation.

The mass trapping system using pheromone baited trap was more efficient for attractiveness of weevil compare to common pseudo-stem trap. Therefore, a preliminary study of controlling banana weevil using biopesticide associated to pheromone baited trap revealed that pheromone trap can optimize biopesticide efficacy in the field causing up to 83.3 and 78.7 % mortality and mycosis rates of adult *C. sordidus*.

Perspectives

Further investigations are needed before the industrialization of active ingredients studied:

- Perform molecular characterization of Cameroon isolates to identify them compare to those studied by other researchers;
- Explore endophytic potential of Cameroonian isolates to other banana and plantain cultivars using different application technology
- carried out an interaction host-fungi modeling under different environmental factors to deduce Cameroonian fungi isolate ecological zone of performance

- Evaluate the insecticidal potential of BIITAC6.2.2-mutant obtained after 30 min exposure under UV-B light.
- assess performance of microbial formulation developed after long-term at -50, -20 and 4 °C.
- Develop seeding technology using entomopathogenic fungi
- Determine performance of kaolin clay formulation as attract and kill strategy using pheromone baited trap in large scale banana and plantain field; and if effective apply in different agro-ecological zone of banana /plantain production.

Recommendations

Based on results and conclusions made in this work, the following recommendations can be done:

- To smallholder we can say that apart from insecticide there are effective tool such as natural enemies (entomopathogenic fungi) that can contribute as an integrated pest management tool to avoid resistance and other deleterious effects due to the intensive use of chemical;
- To researcher: we encourage investigate on safety tool as natural resources (entomopathogenic fungi) against both agricultural and non-agricultural pests;
- To donors, manufacturers and Cameroon governments, find interest to Cameroonian natural resources as entomopathogenic fungi and finance studied and industrial production of mycoinsecticides base in Cameroon isolates which can contribute to improve banana production (to help Cameroon maintain the rank of first producers of banana/plantain in Africa), provide jobs and licence to reduce poverty. The use of kaolin clay, charcoal, wood ash, cassava starch, banana flour and maize for the development of *B. bassiana* can enhance the value chain the mention crops.

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APPENDIX

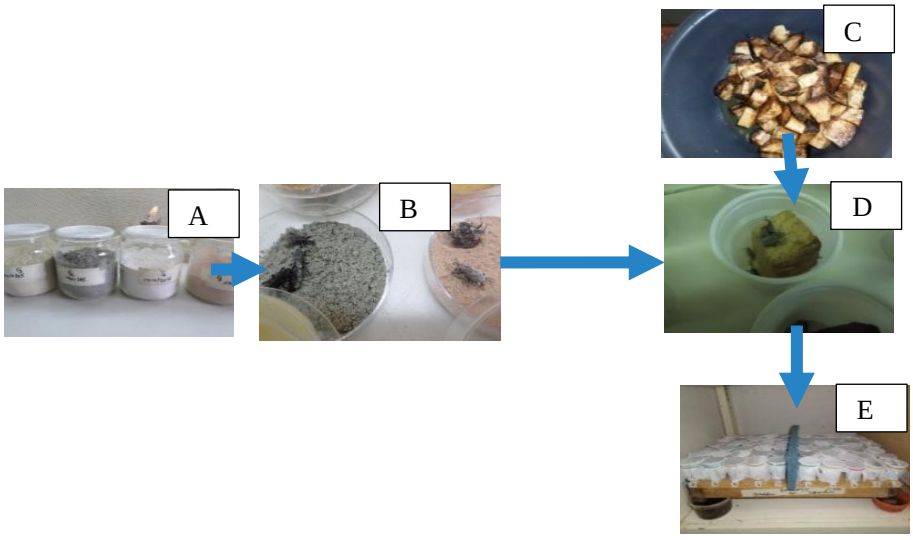
Appendix 1: Origin of the six isolates of *Beauveria bassiana* and *Metarhizium anisopliae* (collected in Cameroon in year 2012) studied.

Speices	Isolate code	Source locality	Geographic coordinates of source	
M. anisopliae	MIITAC11.3.4	Bouidon-Ombessa	N04°35′68.0”	E011°15′81.3”
M. anisopliae	MIITAC6.4.2	Nyassakounou	N04°53′63.3”	E011°25′44.3”
M. anisopliae	MIITAC6.2.2	Nyassakounou	N04°53′35.2”	E011°25′14.2”
B. bassiana	BIITAC10.3.3	Kiki	N04°41′86.7”	E011°10′87.6”
B. bassiana	BIITAC8.1.5	Yangafock	N04°47′02.0”	E011°26′17.5”
B. bassiana	BIITAC6.2.2	Nyassakounou	N04°53′35.2”	E011°25′14.2”

Appendix 2 : Endophytic test

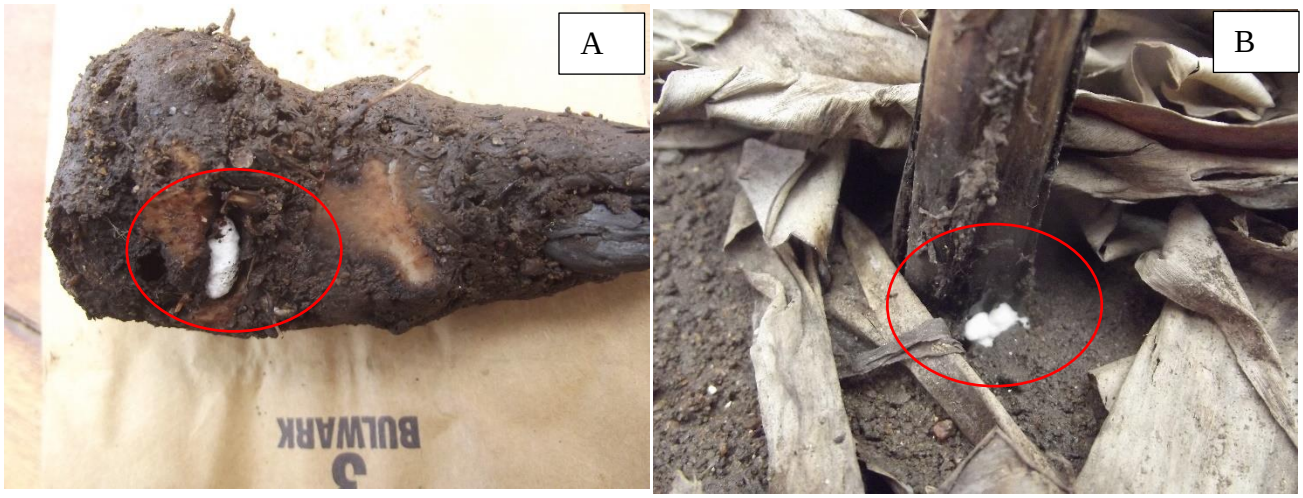
A) corm inoculated; B) Injection of vitroplant; C) Spraying of banana leaves with fungal suspension

Appendix 3: Bioefficacy test procedure in laboratory conditions



A) Formulations; B) inoculation; C) disinfected corm ; D) Insect feeding; E) incubation

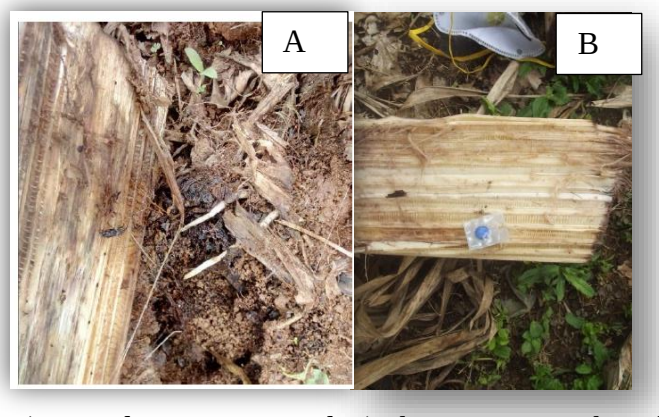
Appendix 4: Banana weevil showing mycosis in greenhouse trial



A) Pupal mycosis and B) adult weevil mycosis

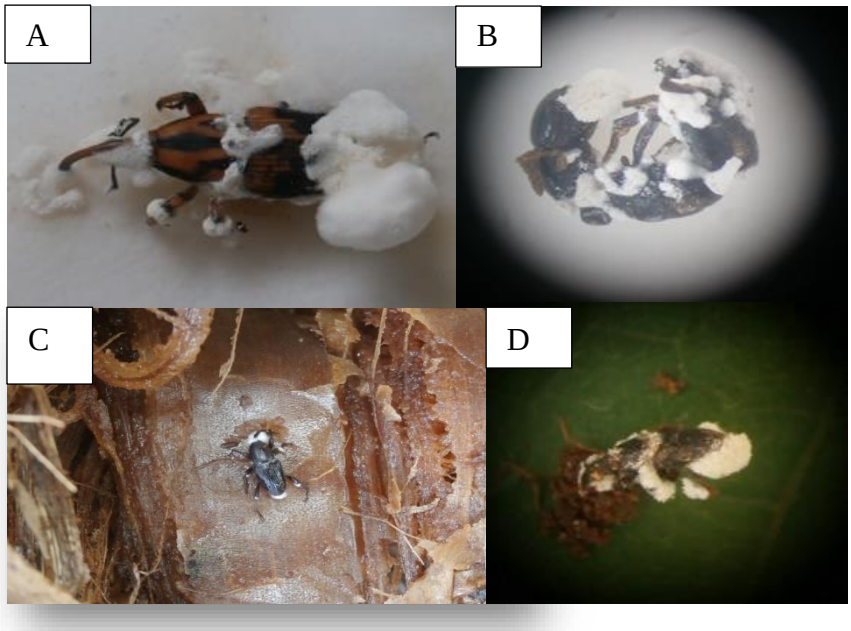
Appendix 5: Biopesticide field trial from establishment to maturity

Appendix 6: Trapping system for mycoinsecticide application combined with pheromone



B) Pseudo-stem trap and D) Pheromone combined to pseudostep trap

Appendix 7: Insects showing fungi out growth after application of mycoinsecticide when combined with pheromone



A) *Metamacius hemipterus*, B) *Pachycondyla tarsata*, C) *Cosmopolites sordidus* and D) *Polytus mellerborgi*

ARTICLES PUBLISHED FROM THE THESIS

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Cosmopolites sordidus (Germar) susceptibility to indigenous Cameroonian *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) isolates

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Abstract

Management of the banana root borer (BRB), *Cosmopolites sordidus* (Germar; Coleoptera: Curculionidae), remains a challenge in banana and plantain production worldwide. Synthetic pesticides remain the most widely used solution while mycoinsecticides are increasingly being recommended. In this study, we selected indigenous isolates of *Beauveria bassiana* and *Metarhizium anisopliae* collected from plantain fields in Cameroon, and tested them in the laboratory for their viability, pathogenicity and virulence against all *C. sordidus* life stages. Of 13 isolates initially screened for spore germination and pathogenicity to adult weevils in conidial suspension of 3.2×10^8 conidia/ml, eight isolates with high to moderate germination and highest weevil mortality were selected for dose–response bioassays with four concentrations per isolate: 3.2×10^2, 3.2×10^4, 3.2×10^6 and 3.2×10^8 conidia/ml. The virulent isolates from adult bioassays were tested with eggs, larva and pupae in conidial suspension of 3.2×10^8 conidia/ml. Isolates performance depended on insect life stage with significantly high pathogenicity and virulence against larval, pupa and adult stages. The *Beauveria* isolate BIITAC6.2.2 caused the highest mortality rates followed by MIITAC1.1.5. Lethal times and lethal concentrations were relatively low for the three *M. anisopliae* isolates and three *B. bassiana* isolates which were the best isolates in almost all insect life stages. Apart from being effective in multiple life stages, these isolates were transmitted horizontally from one stage to another when eggs and pupae were treated. The implication of these findings for integrated management of the BRB, and potential biopesticides development and commercialization are discussed.

KEYWORDS
biodiversity, inoculum transmission, microbial biopesticides, virulence

1 | INTRODUCTION

Banana and plantain are important commodities for food and nutrition security of more than 400 million persons (Paranthaman, Sudha, & Kumaravel, 2012), with about 187 million metric tons produced annually worldwide (FAOSTAT, 2020) and an international banana trade value between US$ 4.5 and 5 billion per year (Arias, Dankers, Liu, & Pilkauskas, 2003). In the central Africa region, banana and plantain together occupy the second rank in crop production, after cassava (FAOSTAT, 2020). Cameroon, with its annual production

of 5.6 million tons, is the 8th producer worldwide and the highest producer in Africa (FAOSTAT, 2020). Several biotic and abiotic constraints, however, continue to challenge banana and plantain production, regardless of region or country.

In central Africa, the major biotic constraints include banana bunchy top disease (BBTD), Banana *Xanthomonas* Wilt (BXW), fungal wilts (Panama disease and Sigatoka) (Blomme et al., 2017; Ngatat et al., 2017), nematodes (Jones, 2000a) and insect pests (Masanza, 2003). The banana root borer (BRB) *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is the most widespread of all insect pest constraints (Gold, Pena, & Karamura, 2001; Okolle, Gagriel, Franklin, Patrick, & Loubana, 2009;Stover, 2000), in addition to the banana aphid *Pentalonia nigronervosa* (Cocquerel; Hemipetera: Aphididae), the vector of banana bunchy top virus, the causal agent of BBTD (Kumar et al., 2011).

Larvae of BRB tunnel through banana and plantain corms leading to several types of damage to banana and plantain (Gold & Messiaen, 2000). These include (a) reduction in planting material availability through pre-mature death of suckers, reduced number of suckers, reduced sucker vigour and development of water suckers; (b) decline in fruit production through stunting of plant growth, delayed fruit maturation, reduced bunch size, and plant toppling resulting in reduction of plantation lifespan and massive toppling of bananas. In the absence of effective control measures, severe infestations of BRB can lead to 100% yield losses, affecting livelihood and food security of vulnerable communities (Koppenhfer, Seshu Reddy, & Sikora, 1994; Muñoz, Cañas, Urrea, & Guarín, 2013; Ocan, Musaka, Rubaihayo, Tinzaara, & Blomme, 2008; Sengooba, 1986; Ysenbrandt, Fogain, Messiaen, & Lang, 2000). In Cameroon, BRB is found in all area of banana and plantain production (Okolle et al., 2009).

Several strategies have been used in banana farming systems for the management of BRB, including the use of various cultural, genetic, chemical and biological methods (Akmal, Freed, Malik, & Gul, 2013; Arinaitwe et al., 2014; Kiggundu et al., 2003b; Mongyeh, Ndamukong, & Okolle, 2015; Osorio-Osorio et al., 2017; Sivirihauma et al., 2017). Due to its cryptic nature, repeated synthetic and systemic insecticides' applications remain, however, the most widely used option for BRB control, increasing the risk of pesticide residues in fruits and leaves (Carvalho, 2017; Kim, Kabir, & Jahan, 2017; Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, & Hens, 2016 ;Paranthaman et al., 2012). Entomopathogenic fungi, with their capacity to cause high BRB mortality and to spread locally in BRB populations, have been considered as a safer alternative to synthetic and systemic insecticides (Hasyim, Azwana, & Syafril, 2009; Nankinga et al., 1999).

The entomopathogens *Beauveria bassiana* ((Bals.) Vuill.; Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* ((Metsch.) Sorokin; Hypocreales: Clavicipitaceae) have been shown to be the most promising microbial agents against adult BRB (Aby et al., 2010; Nankinga et al., 1999; Schoeman & Schoeman, 1999; Tinzaara et al., 2015). There is little experimental evidence of the potential of local isolates of entomopathogenic fungi in BRB microbial control in Central Africa. Furthermore, most studies in other regions of Africa targeted BRB adult stages, and very few of them considered susceptibility of immature stages (Godonou, Green, Oduro, Lomer, & Afreh-nuamah, 2000; Kaaya, Seshu Reddy, Kokwaro, & Munyinyi, 1993). In Cameroon, there have been limited researches in developing biopesticides against BRB Indigenous isolates offer the triple advantage of being adapted to local environmental conditions, suitable for use against local strains of pests and do not require permits for introduction in the country. Forty isolates of *B. bassiana* and *M. anisopoliae* were collected from soils under plantain production in Cameroon using baited method with BRB larvae (Membang, 2013). A recent report by Mahot et al. (2019) assessing the performance of these isolates against the cocoa mirid, *Sahlbergella singularis* Hagl. (Heteroptera: Miridae) demonstrated high pathogenicity of six of the forty isolates.

Several methods have been used for the delivery of entomopathogenic fungi, including application of liquid or powder formulation, through attract-and-kill techniques or plant inoculation as endophytes (Akello, Dubois, Coyne, & Kyamanywa, 2008; Akutse, Maniania, Fiaboe, Van den Berg, & Ekesi, 2013; Backman & Sikora, 2008; Gathage et al., 2016; Lacey & Goettel, 1995; Vega et al., 2007). Knowledge of an entomopathogen's effect on the target organism's mortality rate and lethal time and concentration to kill at least 50% of individuals are fundamental for isolate selection (Bayissa et al., 2016; Kaaya et al., 1993; Lopes et al., 2011; Mahot et al., 2019; Mweke et al., 2018). For a cryptic insect like *C. sordidus* living in corms, the fact that infected insects do not die immediately after infection gives the opportunity for further horizontal transmission of inoculum to different developmental stages and adults living inside the corm is of great importance in terms of durability and dissemination of the epizooties.

The main objective of this study is to evaluate the pathogenicity and virulence of 13 indigenous Cameroonian *B. bassiana* and *M. anisopliae* isolates against all life stages of *C. sordidus* to select isolates for further development and commercialization of biopesticide against the pest. It is expected that if found effective against the pest, integration of the most promising isolate into a fungus-based biopesticide formulation in the country will be faster and cheaper for companies desiring to invest in formulating *B. bassiana* or *M. anisopliae* biopesticide for Cameroon and the larger Central African market for the control of the BRB.

2 | MATERIALS AND METHODS

2.1 | Experimental site

The experiments were conducted under laboratory conditions of 25 ± 1°C, 70%–80% relative humidity (RH) and total darkness, in the laboratory of the International Institute of Tropical Agriculture (IITA) in Yaoundé, Cameroon (N03°51′84″, E11°27′76″).

2.2 | Banana root borer colony maintenance

Adults BRB were collected from naturally infested banana fields at IITA-Cameroon using pseudo-stem traps (Tinzaara, Gold, Dicke, Van Huis, & Ragama, 2011). These weevils were confined in an aerated plastic container (40 mm × 14 mm) with disinfected rhizomes of the local plantain variety Elat (AAB) as food. Rhizomes were washed with tap water, then disinfected by dipping in 1% sodium hypochlorite and rinsed thrice in sterile distilled water. The first 2 weeks in the laboratory were considered as quarantine period during which collected insects were inspected daily for possible occurrence of infection prior experimental setup. The dead insects were incubated on moist filter paper in Petri dishes for mycosis observation. None of the adult BRB at that stage showed any signs of fungal infection. The remaining living weevils were divided into two groups of equal number of adults which formed the cohorts of our study. One cohort was directly used in the experiment with adults. The second cohort served for mass rearing where eggs, larvae and pupae were harvested for subsequent experiments. For this purpose, adults were placed in containers with a trimmed plantain sucker (variety Elat) for weevil multiplication, following method of Musabyimana, Saxena, Kairu, Ogol, and Khan (2001) and Tinzaara et al. (2011). Eggs laid by second cohort adult BRB were harvested daily. The eggs were then transferred into a Petri dish lined with moist filter paper (Night, Gold, & Power, 2010) to conserve them in incubation chamber at conditions described above. Larvae obtained from hatched eggs were transferred daily into a plastic container with crushed plantain corm as larval food. To obtain crushed plantain corm, rhizome was trimmed, disinfected with sodium hypochlorite 1% and rinsed three times before mashing in a blender (Severin, KM 3881 made in Germany). Larval food was changed twice per week. One-day-old weevil 5th instar larvae and pupae were harvested from the containers as needed for the various bioassays.

2.3 | Entomopathogenic fungi cultures

The 13 isolates of entomopathogenic fungi used in this study were obtained from the IITA-Cameroon fungi collection germplasm: seven *B. bassiana* isolates and six *M. anisopliae* isolates (Table 1). All the isolates were obtained from bait insect method (Meyling, 2007) in soil samples collected in Cameroon during various banana field surveys. The fungal isolates were identified based on Humber (2012) identification key (Membang, 2013). Information on isolates collection is provided in Table 1 (Membang, 2013). They were first re-isolated from adult weevils and cultured on potato dextrose agar (PDA) for 21 days at 25 ± 1°C in dark and at 70%–80% RH. Fungal conidia of each isolate were scrapped and suspended in 10 ml Tween 80 solution. Conidia concentrations were then quantified using haemocytometer under a microscope at magnification 40×. The suspensions were then diluted to obtain the needed concentrations.

All bioassays were carried out through artificial infection of 5th instar larvae, pupae and adults using immersion method. Conidia germination was evaluated by spreading conidial suspension each isolate, label 3.2×10^6 on PDA plates using inoculation loop. The plates were sealed with parafilm, and incubated for 16 hr at 25 ± 1°C in the dark (Table 1). Percentage germination was determined by counting 400 conidia per plate with four repetition by isolate. Conidia showing longer germ tube than normal conidia were considered as an indicator of spore viability.

TABLE 1 Source localities and conidia germination rate (mean ± *SE*) of 13 isolates of *Beauveria bassiana* and *Metarhizium anisopliae* 16 hr post-incubation on PDA at 25°C

Species	Isolate code	Source locality	Geographic coordinates		Germination (%) (Mean ± SE)
M. anisopliae	MIITAC11.3.4	Bouidon-Ombessa	N04°35′68.0″	E011°15′81.3″	99.7 ± 0.08a
M. anisopliae	MIITAC8.1.2	Yangafock	N04°47′02.0″	E011°26′17.5″	98.8 ± 0.46a
M. anisopliae	MIITAC6.4.2	Nyassakounou	N04°53′63.3″	E011°25′44.3″	99.8 ± 0.08a
M. anisopliae	MIITAC6.2.2	Nyassakounou	N04°53′35.2″	E011°25′14.2″	99.4 ± 0.16a
M. anisopliae	MIITAC5.3.5	Talba	N04°36′71.4″	E011°29′64.4″	99.2 ± 0.22a
M. anisopliae	MIITAC1.1.5	Atinodzoe	N03°48′14.9″	E011°22′70.8″	99.3 ± 0.43a
B. bassiana	BIITAC10.3.3	Kiki	N04°41′86.7″	E011°10′87.6″	58.7 ± 2.38e
B. bassiana	BIITAC10.2.2	Bep Kiki	N04°40′56.0″	E011°07′56.4″	93.8 ± 0.36b
B. bassiana	BIITAC8.2.5	Yangafock	N04°46′77.3″	E011°26′36.3″	78.4 ± 1.33d
B. bassiana	BIITAC8.1.5	Yangafock	N04°47′02.0″	E011°26′17.5″	85.8 ± 0.79c
B. bassiana	BIITAC6.4.4	Nyassakounou	N04°53′63.3″	E011°25′44.3″	26.3 ± 0.68f
B. bassiana	BIITAC6.2.2	Nyassakounou	N04°53′35.2″	E011°25′14.2″	75.8 ± 1.45d
B. bassiana	BIITAC4.2.5	Nguete	N04°26′33.0″	E011°34′90.4″	73.0 ± 0.43d

Note: Means followed by the same letter in the same column are not significantly different with Tukey's HSD test, α = 5%. Isolates were collected from seven localities in Cameroon in the year 2012.

2.4 | Pathogenicity and virulence test on adults

Live adult *C. sordidus*, collected from the rearing units were first sterilized with 1% sodium hypochlorite and rinsed three times in sterile distilled water. The adults were then immersed individually for ~30 s in 1 ml conidial suspension of 3.2×10^8 conidia/ml of each isolate, as described by Hasyim et al. (2009). Control insects were also dipped for ~30 s but in a solution of Tween 80 (0.1% v/v), prepared with sterile distilled water. Treated insects were transferred to sterile Petri dishes covered with cheese cloth and starved for 24 hr before food (20 g crushed plantain corms) was added. Ten treated insects were grouped as a replicate and three replicates for each treatment (each fungus isolate). The dishes were incubated in an insectarium maintained at 25 ± 1°C in darkness and 70%–80% RH. Weevils were individually inspected for dead every 3 days for 36 days (Kaaya et al., 1993; Lopes et al., 2011). Dead weevils were removed and disinfected with 1% of hypochlorite as described previously and incubated on humid filter paper to assess fungal growth.

2.5 | Dose–response

Four *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2, MIITAC1.1.5 and MIITAC6.4.2) and four *B. bassiana* isolates (BIITAC6.2.2, BIITAC8.1.5, BIITAC10.3.3 and BIITAC10.2.2) with mortality rate above 65% and relative short LT_{50} were selected for dose–response assays. Each isolate was prepared according to the direct enumeration procedures (Inglis, Enkerli, & Goettel, 2012) and adjusted at four concentrations: 3.2×10^2, 3.2×10^4, 3.2×10^6 and 3.2×10^8 conidia/ ml (Hasyim et al., 2009). Disinfected adult *C. sordidus* were treated by immersion method following the same process as described above. Control insects were treated with Tween 80 solution (0.1%v/v). Each concentration level consisted of 10 inoculated insects in each of the three replicates. The dishes containing the treated insects were transferred and maintained as described earlier. Weevil mortality was recorded every 3 days during the experimental period (36 days), and weevil was disinfected with 1% sodium hypochlorite and rinsed thrice in sterile distilled water before incubation on humid filter paper for mycosis confirmation.

2.6 | Pathogenicity and virulence on immature stages

For the study of fungal isolates pathogenicity and virulence on immature stages, eggs of less than 24-hr-old, 5th-instar larvae and 1-day-old pupae, the three most potent *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2) and *B. bassiana* isolates (BIITAC6.2.2, BIITAC8.1.5 and BIITAC10.3.3), which showed relatively low LC_{50} or LC_{90} values were used.

Newly laid eggs (<24 hr old) from the weevil culture were used in the egg bioassay. Collected eggs were disinfected with 1% sodium hypochlorite and rinsed three times in sterile distilled water before immersing them in a conidial suspension. A batch of ten eggs was dipped for ~30 s in the fungal suspension at 3.2×10^8 conidia/ml. Control eggs were similarly immersed but in 0.1% solution Tween 80. The egg bioassay consisted of 10 eggs in each treatment, replicated five times. The treated eggs were placed in Petri dishes with humid filter paper, sealed with parafilm, and incubated for 10 days at 25 ± 1°C in darkness. Mortality was abnormal in the control groups which did not allow for proper estimation of egg mortality caused by the fungal isolated. The experiment provided, however information on horizontal transmission of infection from eggs to larvae by relating signs of disease on eggs (i.e. colonization of egg surface by fungi observed under a dissecting microscope) and infection of emerging larvae (Gindin, Levski, Glazer, & Soroker, 2006).

Larvae and pupae were similarly treated as eggs and adults as described above. Fungus-treated and control insects were transferred to sterile cups containing mashed corm as food and incubated in a growth chamber at 25 ± 1°C with constant darkness; and 70%–80% RH. Weevil mortality was monitored every 2 days for a period of 14 days for larvae and 10 days for pupae. Each treatment consisted of five inoculated larvae or pupae per treatment, replicated five or six times respectively. Dead weevils were disinfected as described above and incubated on humid filter paper for the fungal growth confirmation. Mortality and mycosis were recorded in both larvae and pupae bioassays, in addition to mortality and mycosis of emerged adult mycosis in the pupae bioassay (Gindin et al., 2006).

2.7 | Data analysis

Data obtained from the experiment were statistically analysed with R software version 3.4.3. Cumulative mortality rates were corrected using Abbott's formula (Abbott, 1925). Arcsine or square root transformation was used to correct error distributions and normality of germination, corrected mortality and mycosis rates of adults, eggs, larvae and pupae. The percentage of emerged larva and emerged adult BRB was used as response variables in univariate 1-factor (isolate) ANOVA after arcsine or square root transformation of the response variables to correct for heteroscedasticity inherent in our type of data. Where significant factor *F*-tests ($p < .05$) were found, means were separated with Tukey Honestly Significant Difference (HSD) test at $\alpha = .05$ using R software version mentioned above. The values of LT_{50}, LT_{90}, LC_{50} and LC_{90} of the isolates were estimated at 95% confidence limits (CL) using probit analysis with Package "ecotox" using the same version of R software (Wheeler, Park, & Bailey, 2006). Insect mortality was analysed between doses for each isolate using Kaplan–Meier survival analysis (log-rank method) using JMP 8.0.2 software.

3 | RESULTS

3.1 | Conidia viability

Germination rates of *Metarhizium* isolates were significantly higher than those obtained for *Beauveria* isolates ($F = 308$; $df = 12$; $p < .001$). *Metarhizium* isolates had similar conidia viability while *Beauveria* isolates could be grouped into three categories of conidia viability: >85% (BIITAC10.2.2 and BIITAC8.1.5), 73%–78% (BIITAC8.2.5, BIITAC6.2.2 and BIITAC4.2.5), and <60% (BIITAC10.3.3 and BIITAC6.4.4) (Table 1).

3.2 | Susceptibility of *Cosmopolites sordidus* adults to entomopathogenic fungal isolates

Mortality rates of adult BRB that were treated with the eight isolates with the high to moderate germination rates are summarized in Table 2. Adult mortality rates varied significantly among the isolates ($F = 7.05$; $df = 12$; $p < .001$). At 36 days post-treatment results showed that *M. anisopliae* isolate MIITAC5.3.5 was the least virulent while *B. bassiana* BIITAC6.2.2 was the most virulent, causing 50% mortality at 3.37 days post-inoculation (Table 2). Similarly, high levels of pathogenicity and virulence were recorded with four *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.4.2, MIITAC6.2.2 and MIITAC1.1.5) and four *B. bassiana* isolates (BIITAC6.2.2, BIITAC8.1.5, BIITAC10.3.3 and BIITAC10.2.2), which were selected for dose–response experiments.

The values of lethal time of 50 and 90% mortality (LT_{50} and LT_{90}) ranged 3.37–31.2 days and 12.5–34.2 days respectively for fungal isolates studied (Table 2). The least LT_{50} values were obtained when adult BRB was inoculated with BIITAC6.2.2, MIITAC11.3.4 or MIITAC6.2.2. The isolate BIITAC6.2.2 had the lowest LT_{90} (12.5 days).

3.3 | Dose–response

Survival analysis showed that there was no significant difference between doses, when *C. sordidus* was treated with BIITAC6.2.2 (log-rank test, $\chi^2 = 5.8$; $df = 3$; $p = .12$), BIITAC10.3.3 (log-rank test, $\chi^2 = 5.1$; $df = 3$; $p = .16$) and MIITAC11.3.4 (log-rank test, $\chi^2 = 5.96$; $df = 3$; $p = .11$). In contrast, survival rate was significantly lower at highest doses when *C. sordidus* was treated with *B. bassiana* isolates BIITAC8.1.5 (log-rank test, $\chi^2 = 32.5$; $df = 3$; $p < .0001$), BITTAC10.2.2 (log-rank test, $\chi^2 = 26.7$; $df = 3$; $p < .0001$) and *M. anisopliae* isolates MIITAC1.1.5 (log-rank test, $\chi^2 = 50.4$; $df = 3$; $p < .0001$), MIITAC6.2.2 (log-rank test, $\chi^2 = 8.7$; $df = 3$; $p = .03$), MIITAC6.4.2 (log-rank test, $\chi^2 = 33.3$; $df = 3$; $p < .0001$).

TABLE 2 Pathogenicity and virulence of 13 *Metarhizium anisopliae* and *Beauveria bassiana* isolates to adult *Cosmopolites sordidus* at the dose of 3.2×10^8 conidia/ml

Fungal Isolates	Mortality (%)	LT_{50} and 95% FL (days)	LT_{90} and 95% FL(days)
Metarhizium anisopliae			
MIITAC11.3.4	85.2 ± 9.70ab	6.18 (3.41–8.60)	34.1 (24.2–64.6)
MIITAC8.1.2	23.2 ± 6.48cd	[a]	[a]
MIITAC6.4.2	84.3 ± 4.63abc	12.2 (10.82–13.58)	34.2 (29.6–41.2)
MIITAC6.2.2	84.7 ± 3.50abc	7.75 (6.07–9.30)	32.9 (26.7–44.2)
MIITAC5.3.5	7.40 ± 7.40d	[a]	[a]
MIITAC1.1.5	92.6 ± 7.40ab	10.4 (8.12–12.5)	23.1 (19.0–31.1)
Beauveria bassiana			
BIITAC10.3.3	68.1 ± 15.70abc	14.2 (9.83–19.0)	[a]
BIITAC10.2.2	76.9 ± 6.48abc	15.3 (13.4–17.2)	[a]
BIITAC8.2.5	50.5 ± 6.01abcd	31.2 (25.8–41.5)	[a]
BIITAC8.1.5	88.4 ± 6.43ab	7.41 (5.43–9.21)	28.76 (29.6–41.2)
BIITAC6.4.4	48.2 ± 24.28bcd	[a]	[a]
BIITAC6.2.2	96.3 ± 3.70a	3.37 (1.82–4.78)	12.5 (9.63–17.2)
BIITAC4.2.5	38.4 ± 3.24bcd	[a]	[a]

Note: Mean mortality followed by same lower-case letters are not significantly different by Tukey's HSD multiple range test at $p < .05$. Lethal time 50 (LT_{50}) and 90 (LT_{90}) (days) ± 95% fiducial limit (FL).

[a]Mortality <50% or 90%.

Result of dose–response at 36 days after treatment showed that each fungal isolate had different lethal concentration (LC_{50}) value. The isolates MIITAC6.2.2 (3.63×10^3conidia/ml), MIITAC6.4.2 (3.78×10^6 conidia/ml), BIITAC8.1.5 (4.88×10^6 conidia/ml), BIITAC10.3.3 (5.09×10^6 conidia/ml) showed relatively low LC_{50} values (Figures 1 and 2; Table 3). The *B. bassiana* isolate BIITAC6.2.2 had the lowest LC_{90} value (3.98×10^3 conidia/ml) (Figure 2 and Table 3). These six isolates were selected for pathogenicity test on eggs larvae and pupae of *C. sordidus*.

3.4 | Susceptibility of *Cosmopolites sordidus* eggs to entomopathogenic fungal isolates

While mycosis was recorded on both eggs and emerged larvae for all fungal isolates tested (Table 4). The entomopathogenic fungal isolates were significantly different regarding egg mycosis rates ($p = .002$), with only the three *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2) being significantly different from control, but not significantly different from *B. bassiana* isolates. Emerged larvae from treated eggs were horizontally infected by fungi even as larvae were transferred on untreated food. Isolates were significantly different in the horizontal infection of larvae ($p < .001$) with the three isolates of *M. anisopliae* being significantly different from control while no difference was found between *B. bassiana* isolates and the control. Combined mycosis was significantly higher for the three *M. anisopliae's* isolates compared to control ($p < .001$).

3.5 | Susceptibility of *Cosmopolites sordidus* larvae to entomopathogenic fungal isolates

All isolates were pathogenic to the 5th instar larvae of banana weevil *C. sordidus*, with significant high mortalities in the six isolates tested ($p < .001$). Overall, *M. anisopliae* isolates showed equal or superior virulence compared with *B. bassiana* isolates. The *B. bassiana's* isolate BIITAC10.3.3 and *M. anisopliae* MIITAC11.3.4 were the most virulent isolates, causing 100.0 ± 0.00% mortalities on treated larvae (Table 5). The virulence in the other isolates was also high and

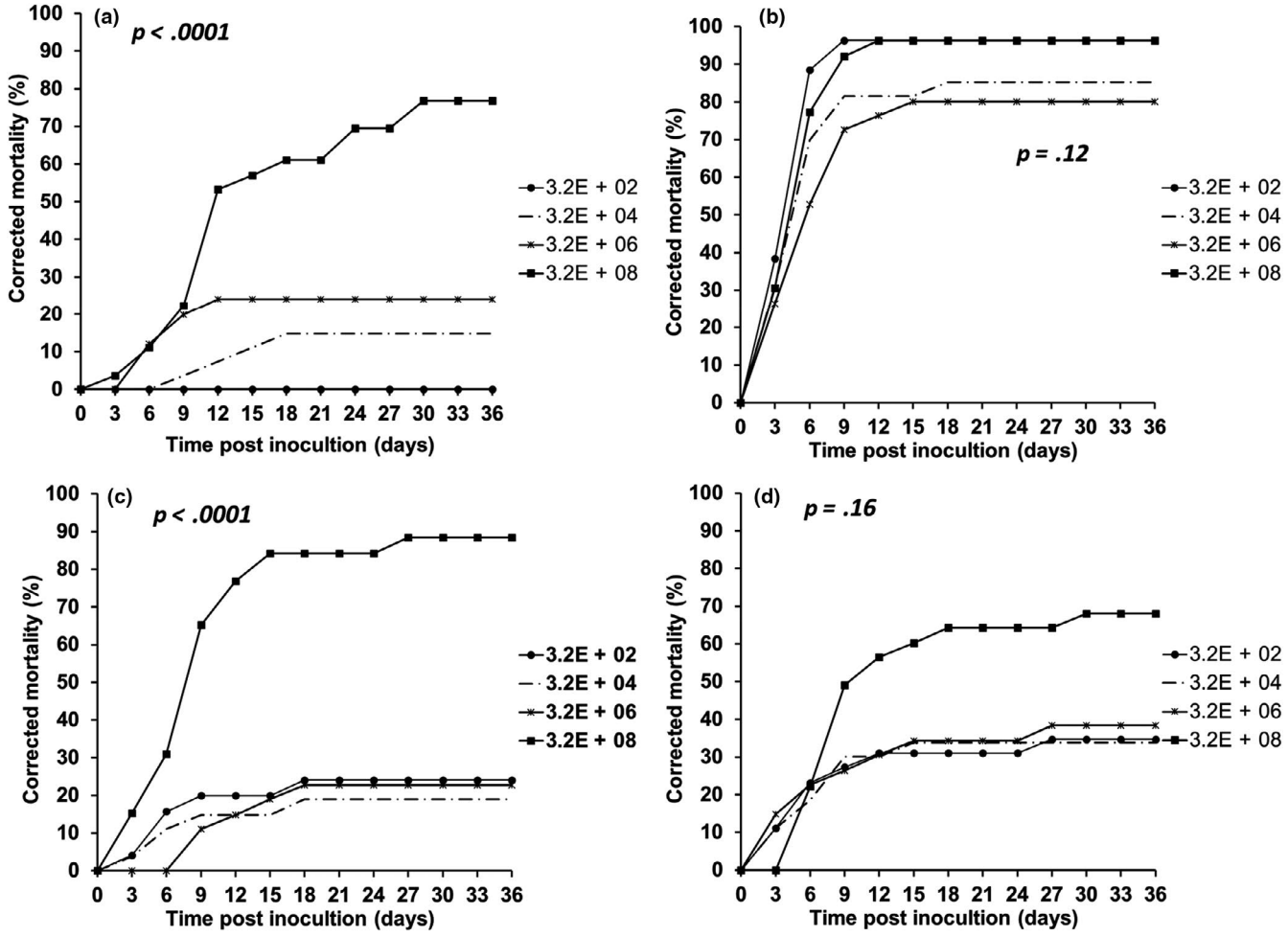

FIGURE 1 Cumulative mortality of adults *Cosmopolites sordidus* treated with four concentrations of each of four *Beauveria bassiana* isolates; (a) BIITAC10.2.2; (b) BIITAC 6.2.2; (c) BIITAC8.1.5; (d) BIITAC10.3.3

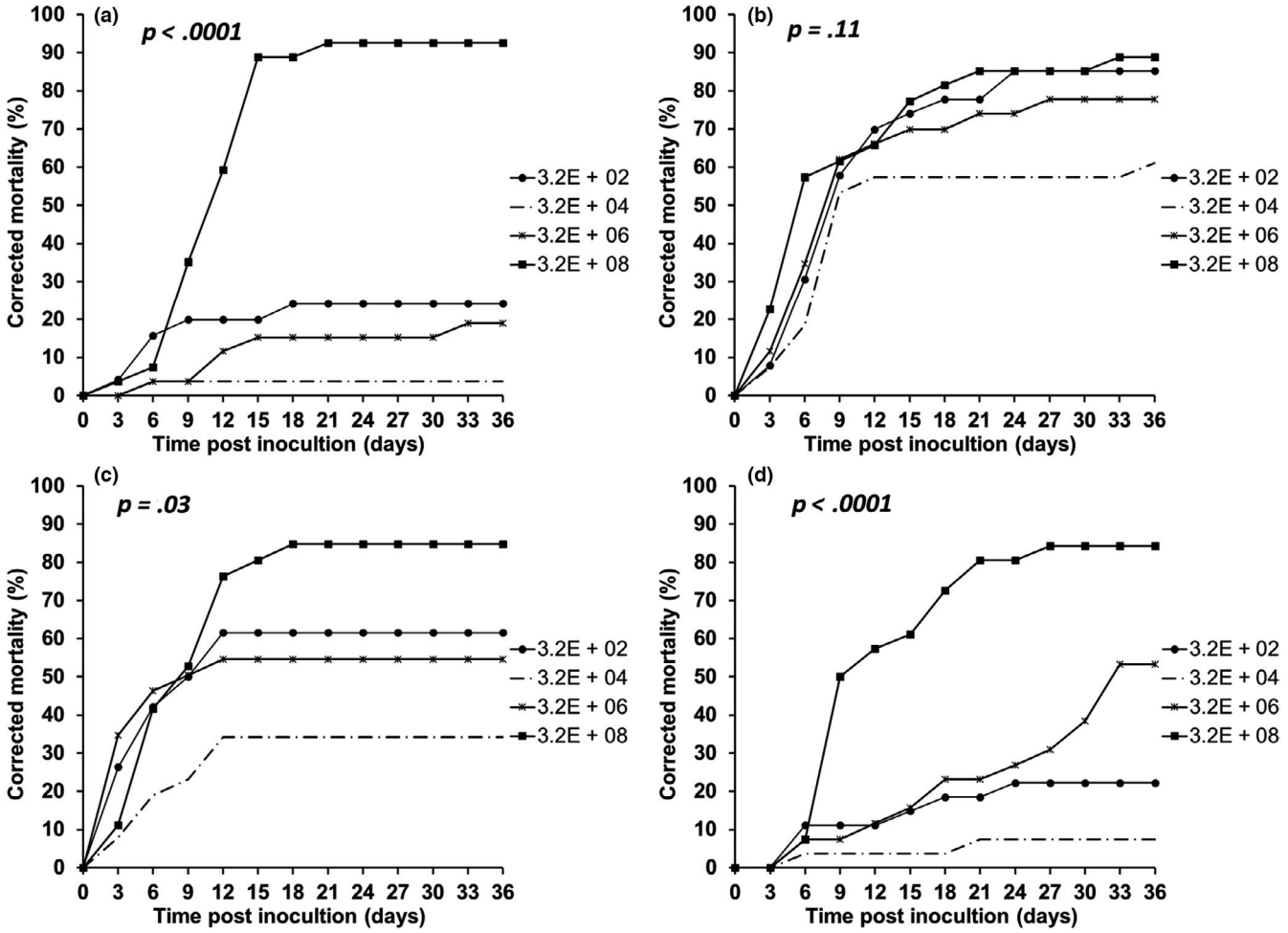

FIGURE 2 Cumulative mortality of adults *Cosmopolites sordidus* treated with four concentrations of each of the four *Metarhizium anisopliae* isolates: (a) MIITAC1.1.5; (b) MIITAC11.3.4; (c) MIITAC6.2.2; (d) MIITAC6.4.2

TABLE 3 Virulence of eight *Beauveria bassiana* and *Metarhizium anisopliae* isolates against adult *Cosmopolites sordidus*, 36 days' post-inoculation

Fungal Isolates	LC_{50} (conidia/ml) (95% FL)	Slopes	Chi-square
Metarhizium anisopliae			
MIITAC6.4.2	3.78×10^6 ($2.62 \times 10^4 \pm 1.66 \times 10^9$)	0.33 ± 0.019	329.25
MIITAC6.2.2	3.63×10^{3a}	0.12 ± 0.019	168.5
MIITAC1.1.5	8.20×10^{6a}	0.32 ± 0.02	683
Beauveria bassiana			
BIITAC10.3.3	5.09×10^{6a}	0.13 ± 0.017	196.68
BIITAC10.2.2	1.98×10^7 ($3.06 \times 10^6 \pm 2.99 \times 10^8$)	0.49 ± 0.03	146.6
BIITAC8.1.5	4.88×10^6 ($4.85 \times 10^4 \pm 4.90 \times 10^{12}$)	0.28 ± 0.018	315.77

[a]Unable to estimate lower and upper limits at 95% fiducial limit from the data.

ranged from 68.1 ± 9.21 to 95.8 ± 4.17%. The values of LT_{50} and LT_{90} ranged from 3.02 to 7.76 days and 7.33 to 9.08 days, respectively. The three *M. anisopliae* isolates and BIITAC10.3.3 had the shortest LT_{50} and LT_{90}. Results of mycosis tests ($p < .001$) (Table 5) revealed approximatively the same trend as mortality rates.

3.6 | Susceptibility of *Cosmopolites sordidus* pupae to entomopathogenic fungal isolates

When the banana weevil's pupae were treated with the different entomopathogenic isolates, pupal mortality and mycosis rate

TABLE 4 Effect of egg treatment with *Beauveria bassiana* and *Metarhizium anisopliae* on egg mycosis and emerged larvae survival (Mean ± SE)

Isolates	Eggs mycosis (%)	Emerged larva mycosis (%)*	Combined mycosis (%)
Beauveria bassiana			
BIITAC10.3.3	10.0 ± 3.10ab	50.0 ± 22.4abcd*	26.0 ± 10.3bc
BIITAC6.2.2	6.00 ± 4.00ab	14.7 ± 9.04cd	12.0 ± 5.83c
BIITAC8.1.5	20.0 ± 10.5ab	33.3 ± 14.9bcd	30.0 ± 14.5bc
Metarhizium anisopliae			
MIITAC11.3.4	32.0 ± 10.2a	100 ± 0.0a	96.0 ± 4.00a
MIITAC6.2.2	26.0 ± 2.44a	70.2 ± 12.5abc	68.0 ± 6.70ab
MIITAC6.4.2	36.0 ± 9.27a	80.0 ± 20.0ab	68.0 ± 16.9ab
Control	0b	0d	0c
	$F = 4.70$; $df = 6$; $p = .002$	$F = 6.81$; $df = 6$; $p < .001$	$F = 13.15$; $df = 6$; $p < .001$

*Larvae here were not treated but received the infection horizontally from eggs treated. Means followed by the same letter in the same column are not significantly different with Tukey HSD test, $\alpha = .05$.

TABLE 5 Pathogenicity and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates at 3.2×10^8 conidia/ml at against 5th instar larva of *Cosmopolitus sordidus*, 14 days' post-inoculation

Fungal Isolates	Mortality (%)	LT_{50} and 95% FL (days)	LT_{90} and 95% FL (days)	Mycosis (%)
Beauveria bassiana				
BIITAC10.3.3	100.00 ± 00a	4.19 (3.50–4.82)	9.08 (7.77–11.23)	60.00 ± 8.94b
BIITAC6.2.2	70.56 ± 11.17bc	7.76 (6.50–9.32)	*	80.55 ± 9.04ab
BIITAC8.1.5	68.05 ± 9.21c	6.07 (4.51–7.75)	*	75.00 ± 12.00ab
Metarhizium anisopliae				
MIITAC11.3.4	100.00 ± 00a	4.10 (3.51–4.65)	7.33 (6.41–8.81)	100.00 ± 00a
MIITAC6.2.2	95.83 ± 4.17ab	4.03 (3.14–4.83)	8.96 (7.37–11.98)	78.33 ± 8.33ab
MIITAC6.4.2	94.44 ± 5.55ab	3.02 (1.88–3.95)	7.38 (5.66–11.50)	68.17 ± 4.17b
	$F = 5.85$; $df = 5$; $p < .0007$			$F = 3.87$; $df = 5$; $p < .0008$

Note: *Mortality rate < 90 %.

Mean mortality and mycosis followed by same lower-case letters are not significantly different by Tukey's HSD at $p > .05$. Lethal time 50 (LT_{50}) and 90 (LT_{90}) (days) at 95% fiducial limit (FL).

differed significantly between isolates ($p = .002$ and <.0001 respectively). The most virulent isolate was BIITAC6.2.2. BIITAC10.3.3 did not cause any mycosis, while pupal mycosis by other isolates was high and similar. Treated pupae transmitted the fungal inoculum to the emerging adults. The mortality of emerged adult was significantly high when pupae were treated with the three *M. anisopliae* isolates and *B. bassiana's* isolate BIITAC8.1.5 ($p = .002$). Mycosis of subsequent adults was significantly high in the three *M. anisopliae* isolates ($p = .002$) (Table 6). However, combined mortality and combined mycosis rates were high for all isolates except BIITAC10.3.3 (Table 6).

4 | DISCUSSION

Biopesticides are important IPM tools and are safe alternatives to pesticides that increase every year in market of crop protection products (Olson, 2015). *Beauveria bassiana* and *M. anisopliae*, two entomopathogenic fungi mainly used as mycoinsecticides are known to be infectious to various agricultural pests including *C. sordidus*(Maina, Galadima, Gambo, & Zakaria, 2018). Discovering of new and indigenous isolates with high virulence and infectivity to all life stages of the target pest can give added value by reducing biopesticide's production cost and property right as well as increasing

TABLE 6 Mortality and infection rates (Mean ± *SE*) of pupae and freshly emerged adults from pupae treated with *Beauveria bassiana* and *Metarhizium anisopliae* isolates

Isolates	Pupal mortality (%)	Pupal mycosis (%)	Emerged adult mortality* (%)	Mycosis of adults (%)	Combined mortality (%)	Combined Mycosis (%)
Beauveria bassiana						
BIITAC10.3.3	18.25 ± 7.08b	0.00 ± 0.00b	27.74 ± 9.93c	12.50 ± 12.50bc	39.30 ± 10.21b	12.50 ± 12.50b
BIITAC6.2.2	73.41 ± 12.30a	75.00 ± 9.37a	38.90 ± 20.03bc	45.83 ± 20.83ab	95.24 ± 4.76a	79.44 ± 7.22a
BIITAC8.1.5	45.33 ± 9.63ab	49.67 ± 13.75a	86.67 ± 8.16ab	54.00 ± 12.88ab	93.81 ± 3.81a	51.52 ± 8.90a
Metarhizium anisopliae						
MIITAC11.3.4	37.73 ± 8.87b	78.33 ± 9.80a	100.00 ± 0.00a	85.83 ± 6.88a	100 ± 00a	77.18 ± 2.95a
MIITAC6.2.2	38.45 ± 8.60ab	86.11 ± 10.90a	90.83 ± 5.83a	66.67 ± 11.38a	94.76 ± 3.33a	68.94 ± 8.86a
MIITAC6.4.2	41.90 ± 6.14ab	73.61 ± 10.20a	90.74 ± 9.26a	88.90 ± 7.03a	94.44 ± 2.78a	77.85 ± 5.96a
	$F = 4.88$; $df = 5$; $p < .002$	$F = 9.64$; $df = 5$; $p < .001$	$F = 7.32$; $df = 5$; $p = .001$	$F = 5.07$; $df = 5$; $p = .002$	$F = 13.1$; $df = 5$; $p < .001$	$F = 19.8$; $df = 5$; $p < .001$

Note: Means followed by the same letter in the same column are not significantly different with Tukey's HSD test, $p > 5\%$.
*Adults were horizontally infected as a result of pupal artificial infection.

their persistence in the system and significant reduction of conventional pesticides used in banana/ plantain plantations. Most studies on the efficacy of entomopathogenic fungi against *C. sordidus* however focused only on adult stage (Aby et al., 2010; Fogain, Messiaen, & Fouré, 2002; Lopes et al., 2011; Nankinga et al., 1999; Schoeman & Schoeman, 1999), and little is known about potential of indigenous entomopathogenic fungi of Cameroon against *C. sordidus*. The present study assessed Cameroonian indigenous isolates targeting both immature and mature life stages of *C. sordidus* in the scope of biopesticide development, commercialization and use in Central African region.

Metarhizium anisopliae's isolates showed higher germination rates compared to *B. bassiana's* isolates except for BIITAC10.2.2 and BIITAC8.1.5. Germination is a process that can be affected by many environmental abiotic factors as well as biotic parameters such as number of sub-culturing, genetic variability, thickness and/or plurality of membranes covering the conidia that influence the permeability of nutrients necessary for germ tubes formation and growth during germination phase. While a higher viability is important for biopesticide efficacy (Faria, Lopes, Souza, & Wraight, 2015), isolate with moderate viability should not be neglected during screening phase since germination can be improved during formulation process and by adding insect immature stage in the culturing media (Alejo et al., 2018; Mola & Afkari, 2012).

Our study also showed that all the fungi isolates were pathogenic and virulent to all stages of *C. sordidus*. However, the performance of isolates depended on life stages. Out of the 13 isolates studied, six isolates were highly pathogenic to adults while four and five were pathogenic to larva and pupa respectively. Unlike *M. anisopliae* where three isolates tested were highly virulent to all life stages, *B. bassiana* isolates' virulence varied significantly, with BIITAC10.3.3 being the only highly pathogenic isolate against larva and BIITAC6.2.2 and BIITAC8.1.5 for pupa. Compared to the results obtained for the same isolates by Mahot et al. (2019) on cocoa mirid *Sahlbergella singularis* (Haglun; Hemiptera: Miridae), the slopes in present study are lower, suggesting that the response of the BRB's populations exposed to fungi were heterogenous in their response compared to cocoa mirid populations used by Mahot et al. (2019). This results might be due to the fact that adults BRB used for virulence study were initially obtained from the field at unknown ages and acclimatized for the experimentation.

Previous efforts to control BRB using entomopathogenic fungi were mostly focus on using adult stage (Aby et al., 2010; Fancelli Dias et al., 2013; Godonou et al., 2000; González et al., 2018; Lopes et al., 2011; Omukoko, Maniania, Wesonga, Kahangi, & Wamocho, 2011; Tinzaara et al., 2015). Only few studies were interested in immature stages. Nganso, Fansi, and Okolle (2010) found that *B. bassiana* strain GHA (Botanigard) applied on corm in laboratory condition was not effective on adult, eggs and larva of *C. sordidus*. Kaaya et al. (1993) reported susceptibility of both larval and adult stages of *C. sordidus* to Kenyan, Thailand and UK isolates of *B. bassiana* and *M. anisopliae* while Godonou (1999) studied the effect of Ugandan and Kenyan isolates of *B. bassiana* on eggs, larvae and adult. Kaaya et al.(1993) obtained high pathogenicity and virulence against both larvae and adult ranging from 63% to 97% mortalities. Similarly to our findings, they reported that larvae were more susceptible than adult. Godonou (1999) reported low infectivity of fungi to adult stage (27% mortality) compared to immature stages (55% and 60% mortality on eggs and larvae respectively). While assessing the effect of *M. anisopliae* and *B. bassiana* against *Rhynchophorus ferrugineus* (Olivier; Coleoptera: Curculionidae), another cryptic weevil attacking palms, Yasin, Wakil, El-Shafie, Bedford, and Miller(2017) reported higher susceptibility of larval stage than adult as observed in our results on *C. sordidus*. This is, however, the first report of virulence of entomopathogenic fungi against *C. sordidus* pupae.

The successful infection process confirmed with sporulation on dead insects is the secondary source of inoculum generally involved in auto-dissemination and epizootic occurrence which helps in disease outbreak in the field and consequently reduces host population and limit insect pest outbreak (Vega et al., 2007). Infectivity of

immature stages, though known to be responsible of the damages caused to banana and plantain (Gold & Messiaen 2000), has been neglected due to difficulties in the direct application of entomopathogenic fungi against these cryptic instars, compared to adults. However, we hypothesize that epizootics in larvae in the banana corm will most likely result in wider spread of the disease within the corm, coupled with faster halting of damage to the plant compared to mortality of adult stage alone. The indigenous Cameroonian isolates hold therefore a high potential in contributing to environmentally friendly management of the BRB. This will even be of greater importance if they also work endophytically. Further studies are warranted to assess possible endophytic potential of these isolates on different banana cultivars and test the mentioned hypothesis on the performance of the isolates under field condition.

The pathogenicity and virulence of the different isolates tested were significantly different. Out of the 13 isolates screened, only six were found efficient against the BRB. These differences between isolates were found also within the same species. For instance, over six *M. anisopliae* isolates and seven isolates of *B. bassiana* tested, only three isolates in each fungal species were found efficient. This denotes differences between isolates belonging to same species as well as difference between both species. Belonging to the same fungal species is therefore not a guarantee for similar virulence level since difference between isolates can rely on different types and quantity of enzymes and metabolites (which are important virulence determinants) produced by specific strain (Abdelaziz et al., 2018). Similar results have been reported by Ruelas-Ayala, García-Gutiérrez, and Archuleta-Torres, (2013), Cheng et al. (2016) and Yasin et al. (2017) using *B. bassiana* and *M. anisopliae* isolates against other weevils like *Tenebrio molitor* (L.; Curculionidae: Coleoptera), *Sitophilus zeamais* (Motschulsky; Curculionidae: Coleoptera), *Curculio nucum* (L.; Curculionidae: Coleoptera) and *R. ferrugineus*, respectively. For instance, Cheng et al. (2016) while assessing four and two strains of *M. anisopliae* and *B. bassiana* respectively, found difference between isolates of *M. anisopliae* but not between isolates *B. bassiana*.

The six virulent indigenous isolates in the present study against the BRB were recently reported highly effective against cocoa mirid (Mahot et al., 2019). Both *M. anisopliae* and *B. bassiana* are known to be infectious to host of numerous insect orders including Orthopera, Lepidoptera, Dermaptera, Diptera and Coleoptera (Maina et al., 2018). Further studies will be carried out to assess the range of pests that could be controlled using these indigenous Cameroonian isolates and select suitable strains for each pest.

Earlier studies performed to control *C. sordidus* with *B. bassiana* and/or *M. anisopliae* isolates showed contrasting results. Kaaya et al. (1993) found that dry spores of an exotic isolate of *M. anisopliae* from UK was less effective against larvae and adult *C. sordidus* resulting in less than 63% mortality while Aby et al. (2010) reported that dry spores of a local isolate of *M. anisopliae* from Ivory Coast caused up to 100% mortality on adult *C. sordidus*. Our findings are similar to those of Aby et al. (2010) though we used fungal suspension and not dry conidia which can easily be attached on wet insect cuticle.

Pathogenicity test of *B. bassiana* isolates against BRB in previous studies varied from low to high virulence. On adults, mortalities varied from 63% to 97% (Kaaya et al., 1993), 6% to 96.7% (Lopes et al., 2011), 14% to 96% (Fancelli Dias et al., 2013) and 27.2% to 80.22% (González et al., 2018). There was limited effort to develop a biopesticide against BRB in central Africa. However, an exotic strain of *B. bassiana* caused low mortality of adult *C. sordidus* (Okolle et al., 2009). Further studies are required to assess the effect of environmental parameters like temperature, rainfall and relative humidity, photoperiod, UV light, pH, compatibility of isolates with biopesticide formulation ingredients in order to take the present findings to actual optimized development of environmentally friendly biopesticides based on the local Cameroonian isolates against BRB and other agricultural pests as well (Mahot et al., 2019).

Despite regular change of substrate (mashed corm), artificial inoculation of eggs and pupae caused infection and mortality in subsequent stages. This horizontal transmission contributed to high combined mortality and mycosis that varied among isolates. It was pronounced in the three *M. anisopliae* isolates while only one *B. bassiana* isolate resulted in horizontal transmission. This is the first such report of horizontal transmission of entomopathogenic fungi in *C. sordidus*. Entomopathogenic fungi are known to cause disease mostly through horizontal transmission (Godonou et al., 2000; Lopes et al., 2011; Omukoko et al., 2011; Schoeman & Schoeman, 1999). Horizontal transmission is not their primary mode of action, and only a few studies reported horizontal transmission through spray, immersion and food poisoning using entomopathogenic fungi (Asi, Bashir, Afzal, Zia, & Akram, 2013; Ekesi, Maniania, & Lux, 2002; Gindin et al., 2006; Gul, Freed, Akmal, & Malik, 2015). Our findings are, however, similar to those reported by Gindin et al. (2006) in which *M. anisoplie* killed horizontally neonate larva of *R. ferrugineus* when eggs were treated; and Asi et al. (2013) where *B. bassiana* and *M. anisopliae* reduced emergence of adult *Spodoptera litura* (Fabricius; Lepidoptera: Noctuidae) when pupal stage was treated. Horizontal transmission is an added value since it adds to mortality of the directly inoculated stage, and subsequently increasing total mortality. Further studies are warranted to understand the process and/or characteristics underlying the differences between isolate, particularly differences between *M. anisopliae* and *B. bassiana* in their potential to horizontally infect BRB.

Overall, 6 of the 13 screened indigenous Cameroonian isolates were found promising against the BRB; namely, *M. anisopliae* isolates MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2 and *B. bassiana* isolates BIITAC6.2.2, BIITAC8.1.5 and BIITAC10.3.3. Apart from causing high mortality of treated BRB life stages, the six isolates transmitted disease horizontally. Further studies on this horizontal transmission are needed to better understand the process and optimize the use of these isolates. Our study showed that six Cameroonian fungal isolates tested are pathogenic and virulent to multiple stages, an important attribute in disease epidemiology that increases efficacy against host populations. The output of our study also offers an unlimited opportunity, in Cameroon and Central Africa in general, to develop biopesticides against various

pests and in various cropping systems using local isolates. Further studies should test these virulent fungi isolates against non-target organisms to identify their host range, susceptibility to environmental parameters, persistence in field conditions and compatibility with different products used in biopesticide development will enable optimization, production and utilization of the indigenous Cameroonian isolate-based biopesticides to improve yield and livelihood of banana growers while promoting biodiversity and environmental safety. This will further improve income generation in agricultural value chains around climate smart and sustainable intensification of crop production.

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CONFLICT OF INTEREST

No conflict of interest declared.

AUTHOR CONTRIBUTION

GM, ZA, AFK and RH conceived research. GM conducted experiment. ZA, HCM, AFK, KKMF and RH provided research materials, tools and intellectual support during research execution. GM, KKMF and RH conducted statistical analyses. GM, ZA, HCM, AFK, KKMF and RH wrote the manuscript. RH and KKMF secured funding. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Thermal response and horizontal transmission of cameroonian isolates of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* – Candidates for microbial controls of the banana root borer *Cosmopolites sordidus*

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ABSTRACT

Beauveria bassiana and *Metarhizium anisopliae* are two promising microbial agents for biopesticides development against the banana root borer *Cosmopolites sordidus*. In this study, germination, mycelial growth, and sporulation of six local Cameroonian isolates of those two species were assessed under seven different thermal conditions (13, 15, 20, 25, 29 33, and 37 °C) to select thermo-tolerant isolates. The Transmission potential of the thermo-tolerant isolates was determined at 25 ± 1 °C by dipping adult weevils in conidial suspensions (3.2×10^8) conidia/ml and mixing these with uninoculated weevils in different proportions (0, 10, 30 and 50%), in groups of 30, and assessing the spread of the mycosis within the group over 35 d of co-incubation. Incubation temperature and isolates significantly affected germination, mycelial growth and conidial production. All isolates had large thermal tolerance ranges (13 −33 °C) except MIITAC6.4.2 (20−29 °C). Horizontal transmission resulted in mortality of non-inoculated weevils from 4.63 ± 1.77 to 53.3 ± 11.9%. The isolate BIITAC6.2.2 exhibited high auto-dissemination potential and high conidia yield in cadavers. These results demonstrate the potential use of these isolates for biopesticides development against *C. sordidus* in Central Africa.

1. Introduction

Several species and strains of entomopathogenic fungi (EPF) originating from a wide diversity of environments have been used in microbial control of mites and insects (Vega et al., 2012). The entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae,* by their easy mass production on artificial media, are the most widely used myco-insecticides (Jaronski, 2013; Brunner-Mendoza et al., 2019). The virulence and multiple modes of action of these two species contribute to the rarity of resistance among host arthropods and enhances the value of EPF as tools in integrated pest management programs (Butt et al., 2016; Petrisor and Stoian, 2017; Abdelaziz et al., 2018). Specific ecological and biophysical requirements, however, continue to pose serious challenges to the development and utilization of myco-insecticides (Maina et al., 2018).

Numerous studies have shown that abiotic and biotic factors such as temperature, relative humidity, solar radiation, competition with other microbes, and arthropod density and diversity affect the infection process during exposure of the arthropods to the EPFs. The effects of these factors are usually determined through evaluation of the action of environmental variables on biological parameters, fungus virulence, persistence, and dissemination of inoculum in the field of which one or more of these factors can limit

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myco-insecticide use (Nankinga et al., 1999; Vänninen et al., 2000; Inglis et al., 2001; Vega et al., 2012). Among the abiotic factors, temperature is the most critical in both arthropod host biology (e.g., life cycle duration, host activity and consequently host population growth rate) and entomopathogen performance (i.e., fungus growth and pathogenicity) (Katsaruware-Chapoto et al., 2017; Taylor et al., 2018; Alali et al., 2019). Temperature has been linked to inconsistent or reduced field performance of some EPF strains. Thompson and Reddy (2016) even recommended that with a changing climate, greater attention should be placed on the effect of temperature on the efficacy of myco-insecticides. Extreme temperature changes can cause cellular stress or death, and initiate appropriate responses that enable them to survive (Shapiro and Cowen, 2012; Braga et al., 2015). Knowledge of thermal tolerance range is fundamental for conservation biological control and for selection of fungi that can offer good ecosystem service or be manipulated depending on insect habitat (Meylind and Eilenberg, 2007). EPF strains that can tolerate a wide thermal range should be therefore an essential component of the selection of EPF in anticipation of the environmental and ecological challenges of the development of sustainable biopesticide products (Alali et al., 2019). The complexity of climatic and agro-ecological zones in Cameroon known to harbor major agro-ecologies of Africa (Robiglio and Sinclair, 2011; Yengoh et al., 2011) could be an advantage for suitable selection of biocontrol agents that can tolerate a wide range of temperatures and maintain its efficient performance widely.

Banana root borer *Cosmopolites sordidus* (Coleoptera: Curculionidae) is the most destructive insect pest of banana and plantain worldwide. It feeds, develops, and oviposits in the corm and rhizomes of banana plants in the soil. Several *B. bassiana* isolates have been tested for the development of myco-insecticides for BRB control with variable levels of success (Schoeman and Schoeman 1999; Godonou et al., 2000; Lopes et al., 2011; Omukoko et al., 2014). In contrast, *M. anisopliae* though effective has been rarely tested against BRB (Kaaya et al., 1993; Aby et al., 2010).

Poor performance of biopesticides against BRB in the field combined with short environmental persistence and difficulty to reach the target, are major challenges of biopesticide use in banana agro-system worldwide (Grzywacz et al., 2009). The efficacy of EPF depends on its ability to spread in the target insect population, typically through horizontal transmission (or auto-dissemination) from infected to healthy individuals, which generally improves disease persistence and the intensity of epizootics that can limit the target host arthropod population (Vega et al., 2007; Lopes et al., 2011; Sharifiifard et al., 2012). Earlier reports even supported the idea that auto-dissemination potential of EPF can be exploited to increase biopesticide performance under field conditions (Nankinga et al., 1999; Godonou et al., 2000; Nankinga and Moore, 2000). The auto-dissemination potential of EPF targeting BRB has inspired several innovative delivery systems of biopesticide applications including attract-kill strategy where an attractant such as kairomone (phenolic odor of banana and plantain corm or pseudostem) and/or sex pheromone (cosmolure) are combined with EPF to favor inoculum dissemination in host populations (Nankinga et al., 1999; Lopes et al., 2014; Uzakah et al., 2015). To date, the attract-kill strategy has been one of the best biopesticide delivery systems in the field for the control *C. sordidus* (Godonou et al., 2000; Nankinga and Moore, 2000; Tinzaara et al., 2007, 2015; Fanceli et al., 2013; González et al., 2018; Moreira et al., 2017; Opisa et al., 2019). However, conidiogenesis is a highly sensitive process that requires special attention given that it also affects auto-dissemination of the fungus and consequently its persistence in the field, which, in turn, will affect the costs of industrialization for a virulent strain. Ahmad et al. (2016) even suggested that thermal tolerance of EPFs should be considered as the main factor for the industrialization of products that can adapt to various environments.

In Central Africa, very limited efforts have been made to identify and use local EPF strains in the fight against BRB. Recently, Membang (2013) isolated seven *B. bassiana* and 33 *M. anisopoliae* isolates from plantain rhizosphere in Cameroon. Some of these isolates were highly pathogenic to *Sahlbergella singularis* (Mahot et al., 2019), *Nisotra uniformis* (Niyibizi, 2018) and BRB (Membang et al., 2020). Six of the isolates showed high infectivity against multiple life stages of BRB (Membang et al., 2020).

The broad objective of this study is to contribute knowledge that can be used in the development of biopesticides based on locally-adapted strains of *B. bassiana* and *M. anisopliae* for the control of BRB in Central African countries and beyond. In this study, we determined the range of thermal response and horizontal transmission potential of six isolates of *B. bassiana* and *M. anisopliae* that had shown high levels of pathogenicity to BRB (Membang et al., 2020).

2. Materials and methods

The experiments were carried out in the laboratories of the International Institute of Tropical Agriculture (IITA) in Yaoundé, Cameroon (N 03° 51′ 84”; E 11° 27′ 76”).

2.1. Weevil and fungi cultures

A laboratory BRB colony was initiated with adult BRB collected with pseudo-stem traps (Tinzaara et al., 2011) from plantain fields at the experimental farm of IITA-Cameroon. One hundred BRB adults were added to containers housing plantain suckers. Three days after the addition of adults, BRB eggs were removed from the corms and inserted (with fine-tip hairbrush) into small holes in weevil-free corms of young suckers (never infested with BRB). The newly infested suckers were placed in opaque plastic containers (40 × 14 mm D x H) lined at their bottoms with wet filter paper and sterilized river sand and incubated in continuous dark conditions at 25 ± 1 °C. At 45 d post-incubation, BRB adults were hand-harvested for their use in fungus isolate re-isolation and horizontal transmission experiments. Prior to their use in the experiments, adult weevils were disinfected by dipping in 1% sodium hypochlorite solution for about 30 s and rinsed 3 or 4 times with sterile distilled water under laminar flow.

Six EPF isolates, three *B. bassiana* (BIITAC6.2.2, BIITAC8.1.5, and BIITAC10.3.3), and three *M. anisopliae* (MIITAC6.2.2, MIITAC6.4.2, and MIITAC11.3.4), were used (Table 1). The six isolates were identified using the identification key to Humber (2012), the molecular characterization could have been useful but logistically impossible due to COVID-19. These isolates were selected in previous studies (Membang, 2013; Mahot et al., 2019) and were preserved at −80 °C as conidia suspension and PDA plates in the IITA-Cameroon entomopathogen collection. Before the inception of the experiment, all isolates were re-isolated from healthy adult BRB (from our lab colonies), to ensure full potential in viability and virulence, and were cultured on PDA medium (LAB M Limited 1, United Kingdom). Conidia were recovered from pure cultures for subsequent studies.

2.2. Germination, mycelial growth, and conidiation under constant temperatures

Conidia of each isolate were harvested from the surface of 15-d-old pure culture of each isolate and suspended in 0.1% Tween 80. The suspension was then stirred with a vortex mixer and filtered with a sterile cheesecloth before conidia counting under a

Table 1
Origin and code of *Beauveria bassiana* and *Metarhizium anisopliae* isolates studied.

Fungus species	Isolate	Origin	Geographic coordinates	Year of 1st isolation
M. anisopliae	MIITAC11.3.4	Bouidon-Ombessa	N04° 35.680, E011° 15.813	2012
M. anisopliae	MIITAC6.4.2	Nyassakounou	N04° 53.633, E011° 25.443	2012
M. anisopliae	MIITAC6.2.2	Nyassakounou	N04° 53.352, E011° 25.142	2012
B. bassiana	BIITAC10.3.3	Kiki	N04° 41.867, E011° 10.876	2012
B. bassiana	BIITAC8.1.5	Yangafock	N04° 47.020, E011° 26.175	2012
B. bassiana	BIITAC6.2.2	Nyassakounou	N04° 53.352, E011° 25.142	2012

dissecting microscope using a Malassez cell (Marienfeld, Germany). Conidial viability of each isolate was determined by spreading 0.1 ml of conidial suspension with a concentration of 3.2×10^6 conidia/ml on PDA plates using a spatula. Four replicates were established for each isolate. Covered glass Petri dishes were tightly sealed with parafilm and transferred to climate control chambers (Percival Scientific, USA) set at seven constant temperatures (13, 15, 20, 25, 29, 33, and 37 °C), 70–80% relative humidity and total darkness. The seven constant temperatures were chosen to cover the range of temperatures in regions with banana and plantain cultivations worldwide (above 13 °C and under 38 °C) and where BRB occurs (above 12 °C and under 34 °C) (Traore et al., 1993; Duyck et al., 2012). The germination rate of 400 conidia was assessed at 40X magnification (Leica, Germany). Conidia were considered as germinated when the germinal tube became longer than normal conidia (Petlamul and Prasertsan, 2012).

To determine the mycelial growth capacity of each isolate under the seven constant temperatures, a 4-mm colony disc of each isolate was removed from the same growing plate of 3 to 5-d-old pure culture incubated at 25 °C and placed in the center of Petri dishes, similar to the description of this process in Petlamul and Prasertsan (2012). Inoculated plates were sealed with parafilm and transferred for incubation at the same conditions set for conidia germination. There were 4–5 replicates per isolate at each temperature. Colony radial extent (mm) in each plate was measured daily for 21 d across two perpendicular diameters (mm) drawn on the bottom of the Petri plates. Conidia from each plate were harvested by scraping the media surface with a sterile scalpel and suspending the whole colony in 10 ml of 0.1% Tween 80 solution. Conidia yield was estimated by diluting the conidia suspension and counting the number of spores with a hemocytometer under a light microscope at 40X magnification (Petlamul and Prasertsan, 2012).

2.3. Horizontal transmission

Horizontal transmission potential was determined for *B. bassiana* isolates BIITAC6.2.2 and BIITAC10.3.3 and for *M. anisopliae* isolates MIITAC6.2.2 and MIITAC11.3.4, which had the broadest temperature range for germination and sporulation (see results below). The horizontal transmission was evaluated using fungus-treated and control (uninfected) adult weevils in four vector ratios (i.e, percentages of fungus-treated weevils in groups of 30 insects) of 0, 10, 30, and 50%, in each of 4 replications per isolate (Lopes et al., 2011). Fungus-treated weevils were air-dried at room temperature after marking them with white paint (Nr. Igle-147) on their dorsum, then dipping them individually for 30 s in 0.1 ml conidial suspension of each isolate prepared at a concentration of 3.2×10^8 conidia/ml. Control insects were dipped in a solution of Tween 80 for 30 s. Both fungus-treated and control insects were placed at the diagonally opposite corners of a sterile plastic container (7 × 5 cm) containing 20-g pieces of plantain corm as food which was changed at 5-d intervals. Containers were incubated for 35 d in the laboratory (in darkness, at 25 °C, which is the optimal temperature for adult weevil activity (Cuillé, 1950) and 70–80% relative humidity). Dead, non-inoculated insects were removed and incubated on moist filter paper in Petri dishes at the condition mentioned above to favor mycosis development.

To evaluate sporulation, ten cadavers of non-inoculated insects for each isolate, presenting signs of infection by the pathogens after 7 d (i.e., insect from mycosis confirmation test) were randomly collected to determine conidial yield per cadaver. This process consisted of transferring cadavers individually in a 10-ml sterile solution of 0.1% Tween 80 and shake for 3 min using vortex to break down the chain and facilitate dispersion of conidia (Latifian and Rad, 2012). The suspension was then serially diluted, and spores were counted under Haemocytometer Malassez cell to estimate conidial yields.

2.4. Statistical analysis

Percentage germination, mycelial growth and conidial production of each of the six isolates across seven constant temperatures were analyzed separately with a 2-factor analysis of variance using a complete randomized design. The significance of pairwise differences was assessed using Tukey HSD ($P < 0.05$). The response variables were log-transformed [Log (x+1)] where needed to correct the heterogeneity of the error variances inherent in the types of data presented in this manuscript. A Pearson's correlation test was performed to assess the relationship between germination, mycelial growth, and conidial production.

Mortality and mycosis values from the horizontal transmission experiment were arcsine-transformed to correct for the heterogeneity of error variances but since transformed data were still not normally distributed, a non-parametric Kruskal Wallis test was used to assess the effect of isolate on percentage BRB infection and mortality for each vector ratio. Means were separated using the Wilcoxon test at a threshold of 5%. The conidial yield of the four fungal isolates infecting BRB in the horizontal transmission tests was analyzed with 1-factor ANOVA using a complete randomized design. Significance was tested with Tukey's HSD test.

ANOVA and regression analyses were performed with R while non-parametric tests were done using SAS (version 9.2).

3. Results

3.1. Thermal effect on fungal conidia germination on a solid substrate

Conidia germination rate was highly affected by temperature ($F = 787.7$; $df = 6, 209$; $P < 0.001$) and isolates ($F = 67.4$; $df = 5, 209$; $P < 0.001$), and the effect of temperature on conidia germination was significantly different between isolates (temperature x isolate: $F = 34.4$ $df = 30, 209$; $P < 0.001$) (Table 2). Conidia germinated at all temperatures except at 37 °C. Optimal germination rates of *B. bassiana* BIITAC8.1.5 were from 20 to 25 °C, while a wider optimal thermal range from 20 to 29 °C was recorded for BIITAC10.3.3 and BIITAC6.2.2 (Table 2). An even wider optimal range was obtained for

Table 2
Effect of seven constant temperatures on conidia viability (mean % viability ± SE) of six entomopathogenic fungus isolates.

Temperatures	BIITAC10.3.3	BIITAC6.2.2	BIITAC8.1.5	MIITAC11.3.4	MIITAC6.2.2	MIITAC6.4.2
13 °C	10.0 ± 1.15**bA**	6.05 ± 1.70 **dA**	7.55 ± 0.95**bcA**	0 **cB**	4.70 ± 2.03 **cA**	5.10 ± 2.60 **bcA**
15 °C	12.6 ± 0.48**bBC**	6.55 ± 0.77**dD**	24.4 ± 1.61**bA**	2.85 ± 1.16 **bcE**	7.65 ± 0.62b**cCD**	13.6 ± 1.34**bB**
20 °C	92.7 ± 0.54 **aA**	68.5 ± 1.70**bB**	92.5 ± 0.80 **aA**	90.9 ± 0.28 **abcA**	71.5 ± 3.71**abcB**	94.7 ± 0.36 **aA**
25 °C	76.9 ± 10.10**aAB**	88.0 ± 0.69**aAB**	70.2 ± 16.4 **aB**	99.6 ± 0.13 **aA**	99.4 ± 1.17 **abA**	98.1 ± 0.57**aAB**
29 °C	78.4 ± 5.72 **aB**	82.0 ± 3.67**abB**	11.8 ± 1.52**bC**	99.4 ± 0.15 **abA**	99.7 ± 0.24 **abA**	99.7 ± 0.12 **aA**
33 °C	24.9 ± 2.00**bB**	42.7 ± 9.56 **cB**	9.50 ± 1.05 **bcC**	99.6 ± 2.15 **aA**	100 ± 0.00 **aA**	99.4 ± 0.36 **aA**
37 °C	0**c**	0**e**	0c	0 ± 0**c**	0**c**	0**c**
F, P	F = 82.7; P < 0.0001	F = 115.5; P < 0.0001	F = 36.12; P < 0.0001	F = 1196; P < 0.0001	F = 727.8; P < 0.0001	F = 262.9; P < 0.0001

Means followed by same lower (upper) case letter within column (row) are not significantly different with Tukey HSD test, $\alpha = 5\%$ for temperatures (isolates).

the three *M. anisopliae* isolates from 20 to 33 °C (Table 2). Conidia germination rates of all fungal isolates were less than 15% at 13 and 15 °C.

3.2. Effect of temperature on mycelial growth

Similar to conidia germination (i.e., viability), mycelial growth was strongly affected by temperature (F = 3491.9; df = 6, 4058; P < 0.001) and isolate F = 97.81; df = 5, 4058; P < 0.001), and highly significant interactions between temperature and isolate were recorded on mycelial growth (F = 48.80; df = 30, 4058; P < 0.001).

For all isolates tested, mycelial growth was completely absent at 37 °C and below 1 mm/d at 13 °C. The *B. bassiana* isolates, BIITAC8.1.5 and BIITAC6.2.2 produced the largest colonies, particularly at 20 °C (2.97 ± 0.09 and 2.70 ± 0.06 mm/d respectively). All *B. bassiana* isolates displayed optimum mycelial growth at 15–29 °C except BIITAC6.2.2 for which optimum range occurred at 20–29 °C. All the *M. anisopliae* isolates showed optimum mycelial growth between 20 and 29 °C except for MIITAC6.2.2 which has wider optimum growth at 20–33 °C (Fig. 1).

3.3. Effect of temperature on conidial production

Total conidial production by the six tested fungi was highly significantly affected by temperature (F = 1821.6; df = 6, 167; P < 0.001) and isolates (F = 729.5; df = 5, 167; P < 0.001), and highly significant interactions between temperature and isolate were recorded on conidial production (F = 92.4; df = 30, 167; P < 0.001). All three *B. bassiana*'s isolates and one *M. anisopliae* isolate - MIITAC6.2.2 − produced conidia in the range of 13–33 °C compared with *M. anisopliae's* isolates MIITAC11.3.4 and MIITAC6.4.2 which, respectively, produced conidia in the range of 13–29 °C and 20–29 °C (Table 3). The widest optimum range of temperature for the highest conidial production was at 13–29 °C for MIITAC6.2.2, followed by BIITAC10.3.3 (13–25 °C) and MIITAC11.3.4 and BIITAC8.1.5 which had both highest conidial production at 15–25 °C. The isolates BIITAC6.2.2, MIITAC11.3.4 and BIITAC8.1.5, and MIITAC6.4.2 showed narrow optimum temperature range for high conidia yield, 13 and 25 °C respectively (Table 3).

Pearson's correlation analysis showed that there was a strong relationship between germination and mycelial growth (r = 0.75; n = 208; P < 0.001) while there was no correlation between germination and conidial production (r = −0.02; n = 208; P = 0.80), nor between mycelial growth and conidial production (r = 0.05; n = 208; P = 0.50).

3.4. Horizontal fungal transmission in adult C. sordidus at different vector ratios and conidial production

All isolates showed the ability to disseminate from inoculated to non-inoculated insects, but transmission potential varied with vector ratio. When 10, and 30% of adult weevils were treated with fungal isolate, mortalities were significantly different from control ($\chi 2 = 17.72$; df = 4; P = 0.0014 and $\chi 2 = 16.92$; df = 4; P = 0.002

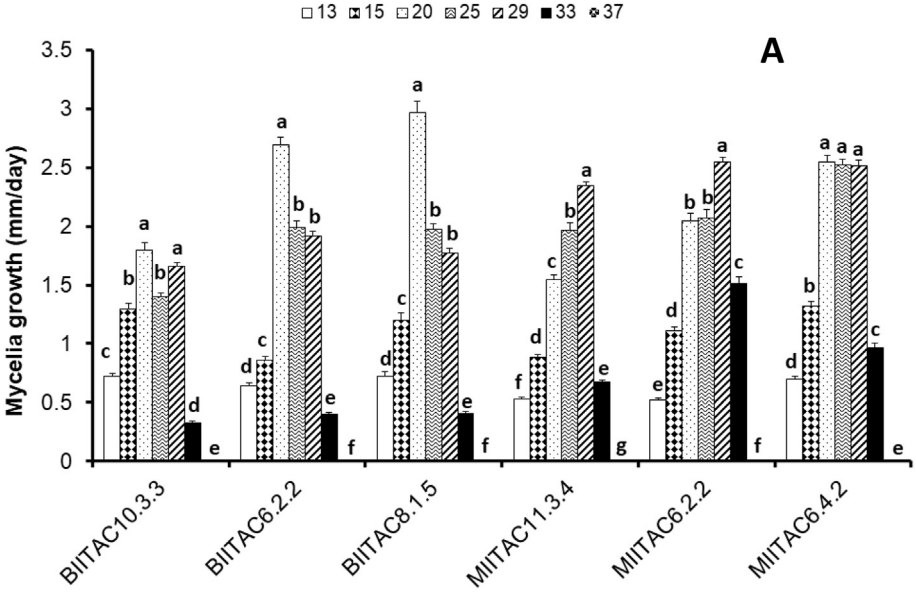

Fig. 1. Mycelial growth of six entomopathogenic fungal isolates incubated on PDA medium at seven constant temperatures (13, 15, 20, 25, 29, 33 and 37 °C).

Table 3
Effect of seven constant temperatures on conidia production (mean unit ± SE) of six entomopathogenic fungus isolates.

Temperature	BIITAC-10.3.3	BIITAC6.2.2	BIITAC-8.1.5	MIITAC-11.3.4	MIITAC-6.2.2	MIITAC-6.4.2
13 °C	(6.00 ± 3.10) x10^8**aB**	(7.50 ± 3.30) x10^9**aA**	(2.50 ± 0.64) x10^8**bB**	(3.52 ± 2.02) x10^7**cC**	(4.35 ± 0.30) x10^7**abBC**	0**dD**
15 °C	(8.61 ± 1.56) x10^8**aA**	(1.62 ± 0.23) x10^9**bA**	(5.43 ± 0.55) x10^8**abA**	(4.10 ± 0.51) x10^8**aA**	(4.93 ± 0.56) x10^7**abA**	0 **dB**
20 °C	(2.01 ± 0.56) x10^9**aA**	(1.88 ± 0.60) x10^9**bA**	(4.45 ± 2.36) x10^8**abB**	(2.83 ± 1.11) x10^8**aB**	(3.35 ± 0.63) x10^8**aB**	(8.18 ± 1.20) x10^5**bC**
25 °C	(2.02 ± 0.80) x10^9**aA**	(2.12 ± 0.65) x10^9**bA**	(2.16 ± 1.10) x10^9**aA**	(2.02 ± 0.70) x10^8**abB**	(1.64 ± 0.32) x10^8**abB**	(6.90 ± 0.22) x10^6**aC**
29 °C	(7.54 ± 5.53) x10^7**bA**	(1.73 ± 0.17) x10^8**cA**	(1.55 ± 0.42) x10^7**cA**	(3.57 ± 1.40) x10^7**bcA**	(1.33 ± 0.23) x10^8**abA**	(1.43 ± 0.45) x10^5**cB**
33 °C	(2.20 ± 0.34) x10^6**bB**	(8.87 ± 1.80) x10^5**dB**	(2.85 ± 0.53) x10^6**dB**	0 **dC**	(6.60 ± 2.27) x10^7**bA**	0 **dC**
37 °C	0**c**	0**e**	0**d**	0**d**	0**c**	0**d**

Means followed by same lower (upper) case letter within column (row) are not significantly different with Tukey HSD test, $\alpha = 5\%$ for temperatures (isolates).

respectively). However, no significant differences were found among the four isolates assessed. At vector ratio 50%, the mortality rates were significantly high with BIITAC6.2.2 followed by the other three isolates which were higher compare to the control ($\chi 2 = 21.40$; df = 4; P = 0.0003). The infection rates of non-inoculated insects was significant high for the four isolates at vector ratio of 10% ($\chi 2 = 14$; df = 4; P = 0.007). A similar trend was observed at vector ratio 30% ($\chi 2 = 12.23$; df = 4, 19; P = 0.016). When 50% of adult weevils were treated, the infection rate of the four isolates were significantly higher than that of the control ($\chi 2 = 20.70$; df = 4, 19; P = 0.0004) with high infection rates for BIITAC6.2.2, BIITAC10.3.3 and MIITAC11.3.4, while MIITAC6.2.2 was not significantly different from MIITAC11.3.4. The mean mortality of control insects was below 1% (0.83%) and none of the dead insects showed mycosis (Fig. 2A and B).

The mean conidia yield by non-inoculated insects was higher for isolate BIITAC6.2.2, ($3.43 \pm 1.90 \times 10^{10}$ conidia/mL compared with the other isolates (F = 59.7; df = 3, 39; P < 0.001)) while BIITAC10.3.3 and MIITAC11.3.4 produced similar numbers of conidia ($1.08 \pm 0.05 \times 10^{10}$ and $7.80 \pm 2.58 \times 10^9$conidia/ml respectively). MIITAC6.2.2 produced the lowest number of conidia ($4.70 \pm 1.61 \times 10^8$conidia/ml) in this study (Fig. 3).

4. Discussion

The success of bio-insecticides based on entomopathogenic fungi (EPF) depends, among other factors, on favorable properties of three successive stages in the growth of entomopathogenic fungi, conidia germination, mycelial growth and conidiogenesis, and their response to environmental variables such as the range of temperatures to which the fungus will be exposed. The three stages determine the ease of inoculum mass production and affect the capacity of the fungus to cause infection and to multiply in the target host. Challenges of environmental conditions have caused field failure and withdrawal of many biopesticides (Maina et al., 2018). Furthermore, the capacity of EPF to disperse within the host populations through horizontal transmission is critical for the ability of the fungus to cause disease epidemics and therefore suppression of the host populations. Studies of the three biological parameters and horizontal transmission are critical in the selection, eventual development, and success of a bio-insecticide.

In this study, we determined the thermal response of three isolates of *B. bassiana* and of *M. anisopliae,* which were isolated from plantain fields in Cameroon (Membang 2013; Membang et al., 2020). This is the first report of the thermal response and

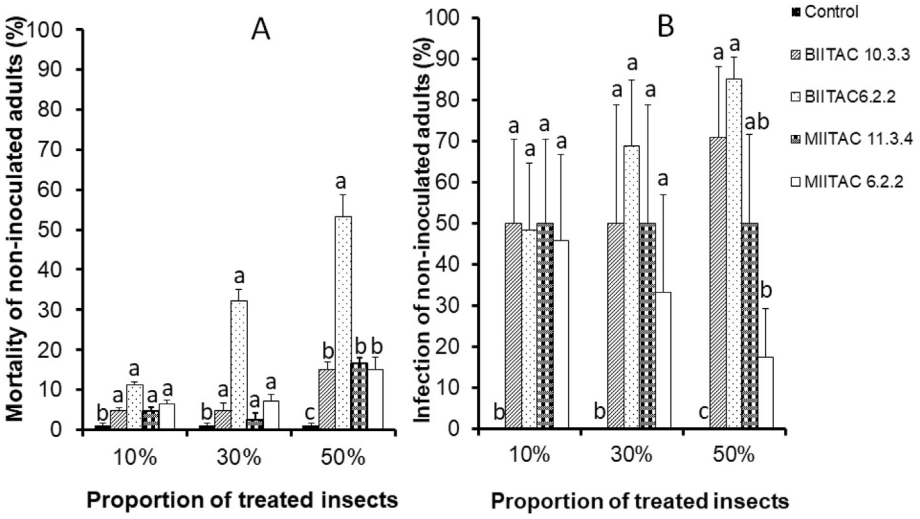

Fig. 2. (A) Percent mortality and (B) mycosis (mean + SE) in experiments on horizontal transmission potential of four entomopathogenic fungi isolates (*B. bassiana* isolates BIITAC10.3.3, BIITAC6.2.2 and *M. anisopliae* isolates MIITAC11.3.4, MIITAC6.2.2) with four increasing levels of vector (inoculated weevils) ratios.

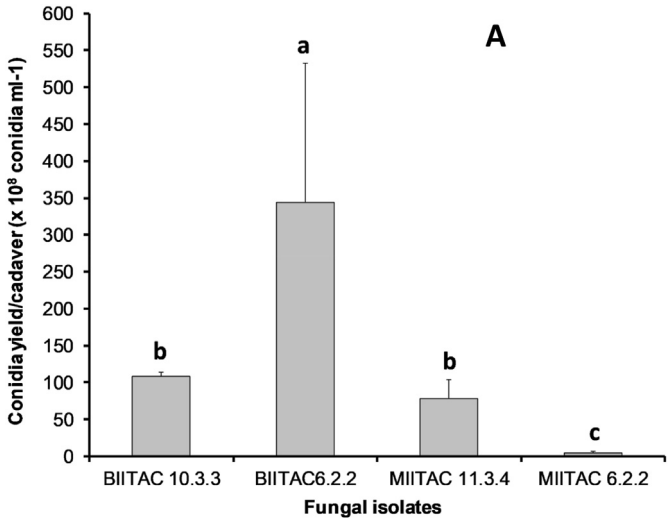

Fig. 3. Fungal conidia yield (mean ± SE) per insect cadaver for four fungal isolates. Means (bars) followed by the same letter are not significantly different (Tukey HSD, $\alpha = 0.05$).

horizontal transmission under laboratory conditions of *B. bassiana* and *M. anisopliae* isolates from Central Africa.

The EPFs used in our study exhibited remarkable adaptability reflected by their ability to germinate, grow and produce conidia at a wide thermal tolerance range with fungal activity inhibited only at 37 °C. The optimum temperature range for maximum conidia germination, high mycelial growth and high conidial production was respectively wide, 20–33 °C, 20–29 °C, and 13–29 °C for *M. anisopliae* and *B. bassiana* isolates studied. This largely agrees with previous studies on the effects of temperature on *B. bassiana* and *M. anisopliae,* which revealed that these fungus species are mostly mesophilic with optima temperature ranges between 20 and 30 °C (Fargues et al., 1992, 1997; Tefera and Pringle, 2003; Lawrence and Khan, 2009; Alali et al., 2019).

Additionally, the thermal response (tolerance and optimum ranges) was fungus isolate-dependent with most isolates tolerating 13–33 °C except MIITAC11.3.4, MIITAC6.4.2 which tolerated, 13–29 °C and 15–33 °C respectively. Earlier reports show that variation in fungi thermal profile could be interspecific (Borisade and Magan, 2014), intraspecific between isolates of same origin (Borisade and Magan, 2014; Heviefo et al., 2019) and also between isolates of different origin (Fargues et al., 1997; Vidal et al., 1997; Teja and Rahaman, 2016; Alali et al., 2019; Heviefo et al., 2019). However, isolates from different origin can also show the same thermal tolerance range, as was the case with *B. bassiana* isolates originating from hot environments in Syria and isolates from Benin which tolerated, 20–30 °C (Alali et al., 2019; Heviefo et al., 2019). EPF strains from the forest can also grow better at low temperatures compared with strains from agricultural fields which could tolerate high temperatures (Augustyniuk-Kram and Kram, 2012). In our study, all the strains were from a forest-savanna transition zone, the microclimate and microhabitat may influence the adaptation potential of a fungus as well as enzymatic, genetics, and molecular regulation that determine the expression or repression of morphological features (Papagianni, 2004; Abdelaziz et al., 2018). Similar to our findings from Cameroon, a report on the thermal sensitivity of five Ethiopian EPF isolates, one *B. bassiana* and four *M. anisopliae,* revealed that *B. bassiana* tolerated 15–30 °C, whereas *Metarhizium* spp. isolates germinated, grew, and sporulated between 15 and 35 °C (Tefera and Pringle, 2003).

The optimum temperature ranges for germination and sporulation obtained in the present study have been favorable for the development of fungus infection in several hosts including BRB (Lawrence and Khan, 2009; Taylor and Khan, 2010; Lopes et al., 2011; Sharififard et al., 2012; Mishra et al., 2015). While testing the thermal effect on the infectivity of two *B. bassiana* strains originating from a warmer region of Brazil, Lopes et al. (2011) demonstrated that some isolates can have a wide thermal range of significantly high infectivity of BRB (21–29 °C), while it is narrow for others (25–29 °C). Similarly, the thermal tolerance range and optimum thermal ranges for fungus *in vitro* growth obtained in the present study is close to both the thermal ranges for growing banana crop (thermal tolerance 19–29 °C and optimum banana growth 26–30 °C) and development of the target pest in our study (thermal tolerance 12 °C to less than 34 °C, optimum ranges 20–30 °C). This means that these fungi can remain active in the banana rhizosphere.

Additionally, all isolates exhibited high activity at 25 °C which is the optimal temperature for adult BRB activity and favorable for disease occurrence (Cuillé, 1950; Lopes et al., 2011*)*. This is important for the dissemination of inoculum in the host through direct contamination, indirect contamination, and secondary transmission of conidia (Vega et al., 2007). It has been exploited for effective control of insect pests using attractants (Nankinga and Moore, 2000; El-Sufty et al., 2010; Tinzaara et al., 2015; Opisa et al., 2019). The wide thermal tolerance of our isolates can also be favorable for the development of a biopesticide product that may withstand wide temperature changes. The tolerance of these isolates to other environmental factors such as humidity, solar radiation, and pH still needs to be assessed.

We observed that the conidial production of *B. bassiana* isolates was 12 times higher compared with *M. anisopliae* isolates**.** Similarly, Lopes et al. (2013), while studying the diversity of indigenous *B. bassiana* and *Metarhizium* spp. in a commercial banana field of Brazil and their virulence toward *C. sordidus*, found that *B. bassiana* produced between 5 and 95 times more conidia per colonized host than indigenous *Metarhizium* spp. isolates. Gandarilla-Pacheco et al. (2012) also reported high sporulation of *B. bassiana* strains at almost all tested temperatures while evaluating conidial production and mycelial growth in solid culture media for native strains of entomopathogenic fungi isolated from citrus-growing areas of México.

The present study results also revealed that conidial production was not directly influenced by mycelial growth and germination. Contrary to our findings, Teja and Rahaman (2016) while testing temperature tolerance of four *M. anisopliae* isolates, obtained a positive correlation between conidia yield and radial growth, and between conidia yield and germination rate. Given that the biological traits studied depend on the nutritive requirement, the type of culture media used, methodology of assessment of conidial production, and fungus specificity may explain the difference between their results and ours. Teja and Rahaman (2016) used PDAY medium, to evaluate conidia yield on 10 mm disc colony for only one fungus species (*M. anisopliae*) incubated at 25–45 °C, while in our case we used PDA, assessed conidial production on the old colony of 21 d using two fungus species (*B. bassiana* and *M. anisopliae*). Those differences imply that detailed studies of individual species and traits are necessary to select suitable microbial agents as a tool for pest management.

Our study showed that the mortality of non-inoculated BRB increased with vector ratio for the more virulent isolate BIITAC6.2.2, with up to 53% mortality at vector ratio 50%. The Mortality of non-inoculated BRB obtained in present study was high compared with those of Schoeman and Schoeman (1999) and Omukoko et al. (2014) who found respectively 27% and 24–26% mortality. Our results are close to those of Lopes et al. (2011) who found 45% mortality of non-inoculated BRB at a vector ratio 50%. A

positive correlation was observed between the proportion of inoculated insects and infection of non-inoculated individuals in studies with entomopathogenic fungi and ants (Pereira and Stimac, 1992), for *C. sordidus* (Lopes et al., 2011), and for *Microcerotermes diversus* (Isoptera: Termitidae) (Cheraghi et al., 2012). The living BRB were effective carriers of inoculum, in contrast to other coleopterans that detect and avoid entomopathogenic fungi (Villani et al., 1994). The number of inoculated insects in the host population may have increased mortality given that the highest mortality was achieved at a high vector ratio 50% (Kocaçevik et al., 2016).

Two studies compared the pathogenicity of the six isolates used in our study and found high virulence of BIITAC10.3.3, BIITAC6.2.2, MIITAC11.3.4, and MIITAC6.2.2 against the cocoa mirid *S. singulris* (Mahot et al., 2019) and high virulence of the six against adult BRB (Membang et al., 2020) respectively causing up to 100.0 ± 00% and 96.7 ± 3.33% mortality. These findings can explain the high potential of transmission, surely related to the level of virulence of the isolates. Ewald (1994) and Myers et al. (1995) reported that efficient transmission of disease by vectors is heavily associated with greater virulence. The high virulence of BIITAC6.2.2 compared with others could be related to pathogenicity factors involved in fungus-host interaction. Pathogenicity factors such as destructive enzymes (protease, chitinase, and lipases) and metabolites can differ in type and amount depending on fungus species and strain (Butt et al., 2016; Petrisor and Stoian, 2017; Abdelaziz et al., 2018).

Conidial production on BRB cadavers was fungus isolate-dependent with significantly higher conidiation for BIITAC6.2.2 compared with all other isolates. The difference in conidial yield between EPF isolates and species was also reported by Bayissa et al. (2016) and Mweke et al. (2018) on aphid cadavers. High conidia yield is important for explosive epizootics, which can lead to disease outbreaks in favorable conditions and consequently lead to reductions in the host population (Myers et al., 1995). The sporulation potential of BIITAC6.2.2 is of great importance for commercialization and the potential for field epizootics.

Author contribution

- Gertrude MEMBANG, Zachee AMBANG, Apollin Fotso KUATE, and Rachid HANNA conceived research
- Gertrude MEMBANG conducted experiment
- Zachee AMBANG, Hermine Claudine MAHOT, Apollin Fotso KUATE, Komi Kouma Mokpokpo FIABOE, and Rachid provided research materials, tools and intellectual support during research execution
- Gertrude MEMBANG, Komi Kouma Mokpokpo FIABOE, and Rachid HANNA conducted statistical analyses
- Gertrude MEMBANG, Zachee AMBANG, Hermine Claudine MAHOT, Apollin Fotso KUATE, Komi Kouma Mokpokpo FIABOE, and Rachid HANNA wrote the manuscript
- Komi Kouma Mokpokpo FIABOE, and Rachid HANNA secured funding
- All authors read and approved the manuscript

Data statement

The dataset can be view using any of the links below.

- DOI: https://doi.org/10.25502/v2pj-g452/d
- CKAN link: http://data.iita.org/dataset/thermal-tolerance-of-cameroonian-fungal-isolates

Declaration of competing interest

No conflict of interest declared.

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