THE UNIVERSITY OF YAOUNDE I
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FACULTY OF SCIENCES
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CENTRE FOR RESEARCH AND TRAINING IN GRADUATE STUDIES IN LIFE, HEALTH AND ENVIRONMENTAL SCIENCES
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CENTRE FOR RESEARCH AND TRAINING UNIT IN LIFE SCIENCES
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UNIVERSITE DE YAOUNDE I
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FACULTE DES SCIENCES
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CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE, SANTE ET ENVIRONNEMENT
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UNITE DE RECHERCHE ET DE FORMATION
DOCTORALE EN SCIENCES DE LA VIE
****

DEPARTMENT OF BIOCHEMISTRY
DEPARTEMENT DE BIOCHIMIE

INSECTICIDAL AND ANTIFUNGAL ACTIVITIES OF *Thymus vulgaris* AND *Cymbopogon citratus* ESSENTIAL OILS AGAINST *Tuta absoluta* AND *Geotrichum candidum* ON TOMATO PRODUCTION IN FOUMBOT-CAMEROON

Thesis presented in partial fulfilment of the requirements for the award of a Doctorat/Ph.D in Biochemistry

BY

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Registration Nº 05X024
Msc in Biochemistry

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Academic year: 2021-2022

THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affaires		**UNIVERSITÉ 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**

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ACADEMIC YEAR 2021/2022

(*by Department and by Grade*)

LAST UPDATED: September 22, 2021

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Vice Dean in Charge of Research and Cooperation: ABOSSOLO Monique, Associate Professor

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Head of Academic Affairs division, Keeping of Terms and Research: AJEAGAH Gideon AGHAINDUM, Professor

1- DEPARTMENT OF BIOCHEMISTRY (BC) (37)

N°	NAME AND SURNAME	GRADE	OBSERVATIONS
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2	FEKAM BOYOM Fabrice	Professor	In service
3	FOKOU Elie	Professor	In service

4	KANSCI Germain	Professor	In service
5	MBACHAM FON Wilfried	Professor	In service
6	MOUNDIPA FEWOU Paul	Professor	**Head of Department**
7	NINTCHOM PENLAP V. épse BENG	Professor	In service
8	OBEN Julius ENYONG	Professor	In service
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14	DJUIDJE NGOUNOUE Marceline	Associate Professor	In service
15	EFFA ONOMO Pierre	Associate Professor	In service
16	EWANE Cécile Annie	Associate Professor	In service
17	MOFOR née TEUGWA Clotilde	Associate Professor	**Insp. Serv. MINESUP**
18	NANA Louise épouse WAKAM	Associate Professor	In service
19	NGONDI Judith Laure	Associate Professor	In service
20	NGUEFACK Julienne	Associate Professor	In service
21	NJAYOU Frédéric Nico	Associate Professor	In service
22	TCHANA KOUATCHOUA Angèle	Associate Professor	In service

23	AKINDEH MBUH NJI	Senior Lecturer	In service
24	BEBEE Fadimatou	Senior Lecturer	In service
25	BEBOY EDJENGUELE Sara Nathalie	Senior Lecturer	In service
25	DAKOLE DABOY Charles	Senior Lecturer	In service

26	DJUIKWO NKONGA Ruth Viviane	Senior Lecturer	In service
27	DONGMO LEKAGNE Joseph Blaise	Senior Lecturer	In service
28	FONKOUA Martin	Senior Lecturer	In service
29	KOTUE TAPTUE Charles	Senior Lecturer	In service
30	LUNGA Paul KEILAH	Senior Lecturer	In service
31	MANANGA Marlyse Joséphine	Senior Lecturer	In service
32	MBONG ANGIE M. Mary Anne	Senior Lecturer	In service
33	Palmer MASUMBE NETONGO	Senior Lecturer	In service
34	PECHANGOU NSANGOU Sylvain	Senior Lecturer	In service
33	FOUPOUAPOUOGNIGNI Yacouba	Assist. Lecturer	In service
34	KOUOH ELOMBO Ferdinand	Assist. Lecturer	In service
35	MBOUCHE FANMOE Marceline Joëlle	Assist. Lecturer	In service
36	OWONA AYISSI Vincent Brice	Assist. Lecturer	In service
37	WILFRIED ANGIE Abia	Assist. Lecturer	In service

2- DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY (ABP) (51)

1	AJEAGAH Gideon AGHAINDUM	Professor	**DAASR /FS**
2	BILONG BILONG Charles-Félix	Professor	**Head of Department**
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4	DJIETO LORDON Champlain	Professor	In service
5	DZEUFIET DJOMENI Paul Désiré	Professor	In service
6	ESSOMBA née NTSAMA MBALA	Professor	**Vice Dean/FMSB/UYI**
7	FOMENA Abraham	Professor	In service
8	KAMTCHOUING Pierre	Professor	In service
9	KEKEUNOU Sévilor	Professor	In service
10	NJAMEN Dieudonné	Professor	In service
11	NJIOKOU Flobert	Professor	In service

12	NOLA Moïse	Professor	In service
13	TAN Paul VERNYUY	Professor	In service
14	TCHUEM TCHUENTE Louis Albert	Professor	**Insp. Serv. Coord. Progr./MINSANTE**
15	ZEBAZE TOGOUET Serge Hubert	Professor	In service
16	BILANDA Danielle Claude	Associate Professor	In service
17	DJIOGUE Séfirin	Associate Professor	In service
18	JATSA BOUKENG Hermine épse MEGAPTCHE	Associate Professor	In service
19	LEKEUFACK FOLEFACK Guy B.	Associate Professor	In service
20	MEGNEKOU Rosette	Associate Professor	In service
21	MONY Ruth épse NTONE	Associate Professor	In service
22	NGUEGUIM TSOFACK Florence	Associate Professor	In service
23	TOMBI Jeannette	Associate Professor	In service

24	ALENE Désirée Chantal	Senior Lecturer	In service
25	ATSAMO Albert Donatien	Senior Lecturer	In service
26	BELLET EDIMO Oscar Roger	Senior Lecturer	In service
27	DONFACK Mireille	Senior Lecturer	In service
28	ETEME ENAMA Serge	Senior Lecturer	In service
29	GOUNOUE KAMKUMO Raceline	Senior Lecturer	In service
30	KANDEDA KAVAYE Antoine	Senior Lecturer	In service
31	MAHOB Raymond Joseph	Senior Lecturer	In service
32	MBENOUN MASSE Paul Serge	Senior Lecturer	In service
33	MOUNGANG Luciane Marlyse	Senior Lecturer	In service
34	MVEYO NDANKEU Yves Patrick	Senior Lecturer	In service
35	NGOUATEU KENFACK Omer Bébé	Senior Lecturer	In service
36	NGUEMBOK	Senior Lecturer	In service
37	NJUA Clarisse Yafi	Senior Lecturer	**Chef Div. UBA**

38	NOAH EWOTI Olive Vivien	Senior Lecturer	In service
39	TADU Zephyrin	Senior Lecturer	In service
40	TAMSA ARFAO Antoine	Senior Lecturer	In service
41	YEDE	Senior Lecturer	In service
42	AMPON NSANGOU Indou	Assistant Lecturer	In service
43	BASSOCK BAYIHA Etienne Didier	Assistant Lecturer	In service
44	ESSAMA MBIDA Désirée Sandrine	Assistant Lecturer	In service
45	FEUGANG YOUMSSI François	Assistant Lecturer	In service
46	FOKAM Alvine Christelle Epse KEGNE	Assistant Lecturer	In service
47	GONWOUO NONO Legrand	Assistant Lecturer	In service
48	KOGA MANG DOBARA	Assistant Lecturer	In service
49	LEME BANOCK Lucie	Assistant Lecturer	In service
50	NWANE Philippe Bienvenu	Assistant Lecturer	In service
51	YOUNOUSSA LAME	Assistant Lecturer	In service

3- DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (PBP) (31)

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2	BELL Joseph Martin	Professor	In service
3	DJOCGOUE Pierre François	Professor	In service
4	MBOLO Marie	Professor	In service
5	MOSSEBO Dominique Claude	Professor	In service
6	YOUMBI Emmanuel	Professor	Head of Department
7	ZAPFACK Louis	Professor	In service
8	ANGONI Hyacinthe	Associate Professor	In service
9	BIYE Elvire Hortense	Associate Professor	In service
10	MALA Armand William	Associate Professor	In service

11	MBARGA BINDZI Marie Alain	Associate Professor	**CT/ MINESUP**
12	NDONGO BEKOLO	Associate Professor	**CE / MINRESI**
13	NGODO MELINGUI Jean Baptiste	Associate Professor	In service
14	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	In service
15	TONFACK Libert Brice	Associate Professor	In service
16	TSOATA Esaïe	Associate Professor	In service

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

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

4- DEPARTMENT OF INORGANIC CHEMISTRY (IC) (32)

1	AGWARA ONDOH Moïse	Professor	**Head of Department**
2	DJOUFAC WOUMFO Emmanuel	Professor	In service
3	Florence UFI CHINJE épouse MELO	Professor	**Rector Univ.Ngaoundere**
4	GHOGOMU Paul MINGO	Professor	**Minister in charge of mission. P.R.**
5	NANSEU Njiki Charles Péguy	Professor	In service
6	NDIFON Peter TEKE	Professor	**CT MINRESI**
7	NDIKONTAR Maurice KOR	Professor	**Vice-Dean Univ. Bamenda**
8	NENWA Justin	Professor	In service
9	NGAMENI Emmanuel	Professor	**Dean FS Uds**
10	NGOMO Horace MANGA	Professor	**Vice Chancellor/U.B**.
11	ACAYANKA Elie	Associate Professor	In service
12	EMADACK Alphonse	Associate Professor	In service
13	KAMGANG YOUBI Georges	Associate Professor	In service
14	KEMMEGNE MBOUGUEM J. C.	Associate Professor	In service
15	KONG SAKEO	Associate Professor	In service
16	NDI NSAMI Julius	Associate Professor	In service
17	NJIOMOU C. épse DJANGANG	Associate Professor	In service
18	NJOYA Dayirou	Associate Professor	In service
19	TCHAKOUTE KOUAMO Hervé	Associate Professor	In service
20	BELIBI BELIBI Placide Désiré	Senior Lecturer	CS/ ENS Bertoua
21	CHEUMANI YONA Arnaud M.	Senior Lecturer	In service
22	KENNE DEDZO GUSTAVE	Senior Lecturer	In service
23	KOUOTOU DAOUDA	Senior Lecturer	In service
24	MAKON Thomas Beauregard	Senior Lecturer	In service
25	MBEY Jean Aime	Senior Lecturer	In service

26	NCHIMI NONO KATIA	Senior Lecturer	In service
27	NEBAH Née NDOSIRI Bridget NDOYE	Senior Lecturer	**CT/ MINPROFF**
28	NYAMEN Linda Dyorisse	Senior Lecturer	In service
29	PABOUDAM GBAMBIE A.	Senior Lecturer	In service
30	NJANKWA NJABONG N. Eric	Assistant Lecturer	In service
31	PATOUOSSA ISSOFA	Assistant Lecturer	In service
32	SIEWE Jean Mermoz	Assistant Lecturer	In service

5- DEPARTMENT OF ORGANIC CHIMISTRY (OC) (40)			
1	DONGO Etienne	Professor	**Vice-Dean/FSE/UYI**
2	GHOGOMU TIH Robert Ralph	Professor	**Dir. BAIF/UDA**
3	NGOUELA Silvère Augustin	Professor	**Head of Department UDs**
4	NYASSE Barthélemy	Professor	In service
5	PEGNYEMB Dieudonné Emmanuel	Professor	**Director/ MINESUP/ Head of Department**
6	WANDJI Jean	Professor	In service

7	Alex de Théodore ATCHADE	Associate Professor	**Vice-Dean/PSAA**
8	AMBASSA Pantaléon	Associate Professor	In service
9	EYONG Kenneth OBEN	Associate Professor	In service
10	FOLEFOC Gabriel NGOSONG	Associate Professor	In service
11	FOTSO WABO Ghislain	Associate Professor	In service
12	KEUMEDJIO Félix	Associate Professor	In service
13	KENMOGNE 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	MVOT AKAK CARINE	Associate Professor	In service

18	NGO MBING Joséphine	Associate Professor	**Sous/Direct. MINERESI**
19	NGONO BIKOBO Dominique Serge	Associate Professor	**Study charge / MINESUP**
20	NOTE LOUGBOT Olivier Placide	Associate Professor	**C.S/ MINESUP**
21	NOUNGOUE TCHAMO Diderot	Associate Professor	In service
22	TABOPDA KUATE Turibio	Associate Professor	In service
23	TAGATSING FOTSING Maurice	Associate Professor	In service
24	TCHOUANKEU Jean-Claude	Associate Professor	**Dean /FS/ UYI**
25	TIH née NGO BILONG E. Anastasie	Associate Professor	In service
26	YANKEP Emmanuel	Associate Professor	In service
27	ZONDEGOUMBA Ernestine	Associate Professor	In service
28	KAMTO Eutrophe Le Doux	Senior Lecturer	In service
29	NGNINTEDO Dominique	Senior Lecturer	In service
30	NGOMO Orléans	Senior Lecturer	In service
31	OUAHOUO WACHE Blandine M.	Senior Lecturer	In service
32	SIELINOU TEDJON Valérie	Senior Lecturer	In service
33	MESSI Angélique Nicolas	Assistant Lecturer	In service
34	MUNVERA MFIFEN Aristide	Assistant Lecturer	In service
35	NONO NONO Éric Carly	Assistant Lecturer	In service
36	OUETE NANTCHOUANG J. L.	Assistant Lecturer	In service
37	TCHAMGOUE Joseph	Assistant Lecturer	In service
38	TSAFFACK Maurice	Assistant Lecturer	In service
39	TSAMO TONTSA Armelle	Assistant Lecturer	In service
40	TSEMEUGNE Joseph	Assistant Lecturer	In service

6- DEPARTMENT OF COMPUTER SCIENCE (CS) (25)

1	ATSA ETOUNDI Roger	Professor	**Chief Div.MINESUP**

2	FOUDA NDJODO Marcel Laurent	Professor	**Head of Dpt ENS/Chef IGA.MINESUP**
3	NDOUNDAM Réné	Associate Professor	In service

4	ABESSOLO ALO'O Gislain	Senior Lecturer	In service
5	AMINOU Halidou	Senior Lecturer	**Head of Department**
6	DJAM Xaviera YOUH – KIMBI	Senior Lecturer	In service
7	DOMGA KOMGUEM Rodrigue	Senior Lecturer	In service
8	EBELE Serge Alain	Senior Lecturer	In service
9	KOUOKAM KOUOKAM E. A.	Senior Lecturer	In service
10	MELATAGIA YONTA Paulin	Senior Lecturer	In service
11	MONTHE DJIADEU Valery M.	Senior Lecturer	In service
12	MOTO MPONG Serge Alain	Senior Lecturer	In service
13	OLLE OLLE Daniel Claude Delort	Senior Lecturer	**Vice Director Enset. Ebolowa**
14	TAPAMO Hyppolite	Senior Lecturer	In service
15	TINDO Gilbert	Senior Lecturer	In service
16	TSOPZE Norbert	Senior Lecturer	In service
17	WAKU KOUAMOU Jules	Senior Lecturer	In service
18	BAYEM Jacques Narcisse	Assistant Lecturer	In service
19	EKODECK Stéphane Gaël Raymond	Assistant Lecturer	In service
20	HAMZA Adamou	Assistant Lecturer	In service
21	JIOMEKONG AZANZI Fidel	Assistant Lecturer	In service
22	MAKEMBE. S . Oswald	Assistant Lecturer	In service
23	MESSI NGUELE Thomas	Assistant Lecturer	In service
24	MEYEMDOU Nadège Sylvianne	Assistant Lecturer	In service
25	NKONDOCK. MI. BAHANACK.N.	Assistant Lecturer	In service

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4	MBANG Joseph	Associate Professor	In service
5	MBEHOU Mohamed	Associate Professor	In service
6	MBELE BIDIMA Martin Ledoux	Associate Professor	In service
7	NKUIMI JUGNIA Célestin	Associate Professor	In service
8	NOUNDJEU Pierre	Associate Professor	**Chief serv. certif. Prog. /FS/UYI**
9	TCHAPNDA NJABO S. B.	Associate Professor	Director/AIMS Rwanda
10	TCHOUNDJA Edgar Landry	Associate Professor	In service

11	BOGSO ANTOINE MARIE	Senior Lecturer	In service
12	AGHOUKENG JIOFACK J. G.	Senior Lecturer	**Chief Cell MINPLAMAT**
13	CHENDJOU Gilbert	Senior Lecturer	In service
14	DJIADEU NGAHA Michel	Senior Lecturer	In service
15	DOUANLA YONTA Herman	Senior Lecturer	In service
16	FOMEKONG Christophe	Senior Lecturer	In service
17	KIKI Maxime Armand	Senior Lecturer	In service
18	MBAKOP Guy Merlin	Senior Lecturer	In service
19	MENGUE MENGUE David Joe	Senior Lecturer	In service
20	NGUEFACK Bernard	Senior Lecturer	In service
21	NIMPA PEFOUKEU Romain	Senior Lecturer	In service
22	POLA DOUNDOU Emmanuel	Senior Lecturer	In service
23	TAKAM SOH Patrice	Senior Lecturer	In service
24	TCHANGANG Roger Duclos	Senior Lecturer	In service
25	TETSADJIO TCHILEPECK M. E.	Senior Lecturer	In service

26	TIAYA TSAGUE N. Anne-Marie	Senior Lecturer	In service
27	BITYE MVONDO E. C.	Assistant Lecturer	In service
28	FOKAM Jean Marcel	Assistant Lecturer	In service
29	LOUMNGAM KAMGA Victor	Assistant Lecturer	In service
30	MBATAKOU Salomon Joseph	Assistant Lecturer	In service
31	MBIAKOP Hilaire George	Assistant Lecturer	In service
32	MEFENZA NOUNTU Thiery	Assistant Lecturer	In service
33	OGADOA AMASSAYOGA	Assistant Lecturer	In service
34	TCHEUTIA Daniel Duviol	Assistant Lecturer	In service
35	TENKEU JEUFACK Y. L.	Assistant Lecturer	In service

8- DEPARTMENT OF MICROBIOLOGY (MIB) (21)

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2	NYEGUE Maximilienne Ascension	Professor	**VICE-DEAN / DSSE**
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4	ASSAM ASSAM Jean Paul	Associate Professor	In service
5	BOYOMO ONANA	Associate Professor	In service
6	KOUITCHEU MABEKU Epse KOUAM Laure Brigitte	Associate Professor	In service
7	RIWOM Sara Honorine	Associate Professor	In service
8	SADO KAMDEM Sylvain Leroy	Associate Professor	In service

9	BODA Maurice	Senior Lecturer	In service
10	BOUGNOM Blaise Pascal	Senior Lecturer	In service
11	ESSONO OBOUGOU Germain G.	Senior Lecturer	In service
12	NJIKI BIKOÏ Jacky	Senior Lecturer	In service
13	TCHIKOUA Roger	Senior Lecturer	In service

14	ESSONO Damien Marie	Assistant Lecturer	In service
15	LAMYE Glory MOH	Assistant Lecturer	In service
16	MEYIN A EBONG Solange	Assistant Lecturer	In service
17	MONI NDEDI Esther Del Florence	Assistant Lecturer	In service
18	NKOUDOU ZE Nardis	Assistant Lecturer	In service
19	SAKE NGANE Carole Stéphanie	Assistant Lecturer	In service
20	TAMATCHO KWEYANG Blandine Pulchérie	Assistant Lecturer	In service
21	TOBOLBAÏ Richard	Assistant Lecturer	In service

9. DEPARTMENT of PHYSIC(PHY) (44)

1	BEN- BOLIE Germain Hubert	Professor	In service
2	DJUIDJE KENMOE épouse ALOYEM	Professor	In service
3	EKOBENA FOUDA Henri Paul	Professor	**Vice-Chancellor. UN**
4	ESSIMBI ZOBO Bernard	Professor	In service
5	KOFANE Timoléon Crépin	Professor	In service
6	NANA ENGO Serge Guy	Professor	In service
7	NANA NBENDJO Blaise	Professor	In service
8	NDJAKA Jean Marie Bienvenu	Professor	**Head of Department**
9	NJANDJOCK NOUCK Philippe	Professor	In service
10	NOUAYOU Robert	Professor	In service
11	PEMHA Elkana	Professor	In service
12	TABOD Charles TABOD	Professor	**Dean FS Univ/Bda**
13	TCHAWOUA Clément	Professor	In service
14	WOAFO Paul	Professor	In service
15	ZEKENG Serge Sylvain	Professor	In service
16	BIYA MOTTO Frédéric	Associate Professor	**GD/HYDRO Mekin**

17	BODO Bertrand	Associate Professor	In service
18	ENYEGUE A NYAM épse	Associate Professor	In service
19	EYEBE FOUDA Jean sire	Associate Professor	In service
20	FEWO Serge Ibraïd	Associate Professor	In service
21	HONA Jacques	Associate Professor	In service
22	MBANE BIOUELE César	Associate Professor	In service
23	MBINACK Clément	Associate Professor	In service
24	NDOP Joseph	Associate Professor	In service
25	SAIDOU	Associate Professor	**Chief of centre/IRGM/MINRESI**
26	SIEWE SIEWE Martin	Associate Professor	In service
27	SIMO Elie	Associate Professor	In service
28	VONDOU Derbetini Appolinaire	Associate Professor	In service
29	WAKATA née BEYA Annie	Associate Professor	*Director/ENS/UYI*

30	ABDOURAHIMI	Senior Lecturer	In service
31	CHAMANI Roméo	Senior Lecturer	In service
32	EDONGUE HERVAIS	Senior Lecturer	In service
33	FOUEDJIO David	Senior Lecturer	**Chief Cell. MINADER**
34	MBONO SAMBA Yves Christian U.	Senior Lecturer	In service
35	MELI'I Joelle Larissa	Senior Lecturer	In service
36	MVOGO ALAIN	Senior Lecturer	In service
37	OBOUNOU Marcel	Senior Lecturer	**DA/Univ Inter Etat/Sangmalima**
38	WOULACHE Rosalie Laure	Senior Lecturer	In service

39	AYISSI EYEBE Guy F. V.	Assistant Lecturer	In service
40	DJIOTANG TCHOTCHOU L. A.	Assistant Lecturer	In service
41	LAMARA Maurice	Assistant Lecturer	In service
42	OTTOU ABE Martin Thierry	Assistant Lecturer	In service

43	TEYOU NGOUPOU Ariel	Assistant Lecturer	In service
44	WANDJI NYAMSI William	Assistant Lecturer	In service

10- DEPARTMENT OF EARTH SCIENCES (E. S.) (42)

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

8	ABOSSOLO née ANGUE Monique	Associate Professor	**Vice-Dean / DRC**
9	BISSO Dieudonné	Associate Professor	**Director/Projet Barrage Memve'ele**
10	EKOMANE Emile	Associate Professor	In service
11	GANNO Sylvestre	Associate Professor	In service
12	GHOGOMU Richard TANWI	Associate Professor	**CD/Uma**
13	MOUNDI Amidou	Associate Professor	**CT/ MINIMDT**
14	NGUEUTCHOUA Gabriel	Associate Professor	**CEA/MINRESI**
15	NJILAH Isaac KONFOR	Associate Professor	In service
16	NYECK Bruno	Associate Professor	In service
17	ONANA Vincent Laurent	Associate Professor	**Chief serv. Mater. Maint /UYII**
18	TCHAKOUNTE J. épse NUMBEM	Associate Professor	**Chef.cell / MINRESI**
19	TCHOUANKOUE Jean-Pierre	Associate Professor	In service
20	TEMDJIM Robert	Associate Professor	In service
21	YENE ATANGANA Joseph Q.	Associate Professor	**Chief Div. /MINTP**

22	ZO'O ZAME Philémon	Associate Professor	**GD/ART**

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

41	FEUMBA Roger	Assistant Lecturer	In service
42	MBANGA NYOBE Jules	Assistant Lecturer	In service

Classification of teaching staff at the faculty of Science of the University of Yaoundé 1

NUMBER OF LECTURERS					
DEPARTMENT	**Professors**	**Associate Professor**	**Senior Lecturer**	**Assistant Lecturer**	**Total**
BCH	8 (01)	14 (10)	13 (05)	05 (02)	**40 (18)**
BPA	15 (01)	8 (06)	18 (05)	10 (03)	**51 (15)**
BPV	07 (01)	9 (01)	8 (06)	07 (01)	**31 (9)**
CI	10 (01)	09 (02)	10 (02)	03 (0)	**32 (5)**
CO	6 (0)	21 (05)	05 (02)	08 (02)	**40 (9)**
IN	2 (0)	1 (0)	14 (01)	08 (01)	**25 (2)**
MAT	2 (0)	8 (0)	15 (01)	09 (02)	**34 (7)**
MIB	3 (0)	5 (03)	06 (01)	06 (02)	**20 (6)**
PHY	15 (0)	14 (02)	09 (03)	08 (03)	**46 (8)**
ST	7 (1)	15 (01)	18 (05)	02 (0)	**42 (7)**
Total	**75 (5)**	**104 (30)**	**116 (31)**	**66 (16)**	**361 (86)**

A total of **361 (**86**)** including:

- Professors **75 (5)**
- Associate Professors **104 (**30**)**
- Senior Lecturers **116 (31)**
- Assistant Lecturers **66** (16**)**

( ) = Number of women **86**

The Dean of the Faculty of Science

Prof. TCHOUANKEU Jean-Claude

DEDICATION

I dedicate this thesis to:

My late father, Mr. TCHONANG David and my mother, Mrs. TCHONANG Denisette;

My husband, Mr. KOUAMEN Rodrigue and childrens, FANDO Cindy, TCHONANG Gloria, KOUAMEN Junior and TCHUISSEU Christ.

ACKNOWLEDGEMENTS

I would like to first of all thank GOD for the support that he provided for the accomplishment of this work.

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
BLAST	Basic Local Alignment Search Tool
DNA	Desoxyribonucleic Acid
Eos	Essential Oils
EPPO	European and Mediterranean Plant Protection Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GC/MS	Gas Chromatography coupled to Mass Spectrometry
GPS	Global Positioning System
ITS	Internal Transcribed Spacer
LSD	Least Significant Difference
MIC	Minimal Inhibitory Concentration
MINADER	Ministère de l'Agriculture et du Développement Rural
NCBI	National Center for Biotechnology Information
NIST	National Institute of Standards and Technology
NS	Not Significant
OD	Optical Density
OECD	Organisation for Economic Co-operation and Development
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
Rdna	Ribosomal Desoxyribonucleic Acid
RPM	Rotation Per Minute

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) yield has recently dropped in Cameroon, failing in supplying the national and neighbouring countries demands. Severe bug infestations, such as *Tuta absoluta*, has favoured tomato rot caused by *Geotrichum candidum*, which is one of the main reasons of decrease productivity. The most common method against damage management is the application of synthetic chemical pesticides, which are well-known for their harm to humans and the environment. Therefore, alternative strategies based on the use of natural products to increase production yield are required. Essential oils, as one of the most reliable biological pesticide sources, have shown promise in terms of efficiency, sustainability, and compatibility with other control measures in the context of Integrated Management Programs. The purpose of this study was to survey on *Tuta absoluta* in Foumbot, the main Cameroonian tomato production basins, and to determine the insecticidal and antifungal activities of *Thymus vulgaris* and *Cymbopogon citratus* essential oils. Data were collected through an interview and field sampling. Interview was performed on the basis of an open and closed response questionnaire with 56 tomato producers from five villages. The quantification of damages was carried out in 19 commercial tomato fields spread in the Foumbot subdivision. The incidence and severity of *Tuta absoluta* infestation were assessed using quadrats drawn in a W pattern and a 1 to 5 rating scale respectively. Furthermore, some factors related to the damage indexes were assessed. Hydro-distillation was used to extract essential oils from *Thymus vulgaris* and *Cymbopogon citratus*, and the oils chemical composition was evaluated using Gas Chromatography coupled with Mass Spectrometry. The insecticidal potential of essential oils was determined by testing their contact and fumigant toxicity against *T. absoluta* larvae. *Geotrichum candidum* was isolated from rotten-infected tomatoes fruits perforated by *Tuta absoluta*. Morphological and molecular traits were used to identify it. Essential oils were tested for antifungal efficacy on pathogens mycelia growth and conidia germination through dilution in solid medium and microdilution in liquid medium, respectively. As results, *Tuta absoluta* occurred in all the surveyed farms in different villages with mean incidence and severity of 93.20% and 4.40 on 1 to 5 rating scale, respectively. The epidemic damages were closely related to the variety and growth stage. The highest values been recorded with hybrid variety (incidence: 88.26%, severity: 4.46) and at the mature fruiting stage (incidence: 97.61%, severity: 4.90). The chemical composition analysis

revealed that *Thymus vulgaris* oil was predominently composed of Thymol (21.53%) and α-Pinene (Dextro) (17.43%) whereas, Neral (34.48%) and Geranial (34.37%) were prominent in *Cymbopogon citratus* oil. Statistical analysis indicated that the both oils exhibited similar knockdown and insecticidal efficiencies through direct contact and fumigation routes. The resulting biological parameters for *Cymbopogon citratus* and *Thymus vulgaris* oils were KD_{50} values of 0.19 µL/mL and 0.59 µL/mL and LD_{50} values of 0.33 µL/mL and 0.61 µL/mL for contact toxicity and KD_{50} of 1.29 µL/mL and 2.57 µL/mL and LD_{50} of 1.48 µL/mL and 3.05 µL/mL for *Cymbopogon citratus* and *Thymus vulgaris* oils respectively for fumigant toxicity. Essential oils exerted larvicidal activity through their anti-acetylcholinesterase effect with similar IC_{50s} value of 2.57 µL/mL and 3.06 µL/mL for *Cymbopogon citratus* and *Cymbopogon citratus* oils respectively. The sequencing of the ITS1-5.8S rDNA-ITS4 gene region and phylogenetic analysis confirmed the identity of the fungi pathogens to be the isolates of *Geotrichum candidum* (*Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3). The antifungal assay has shown that Minimal Inhibitory Concentration values of *Cymbopogon citratus* and *Thymus vulgaris* oils were 0.71 µL/mL and 5.67 µL/mL respectively on mycelia growth of all pathogens. The inhibition of conidia germination revealed that Minimal Inhibitory Concentration was undetermined with *Thymus vulgaris* oil and were 0.71 µL/mL, 1.42 µL/mL and 5.67 µL/mL for *Cymbopogon citratus* oil against *Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3 respectively. The results obtained demonstrate that *Cymbopogon citratus* oil could be used in the control of *Tuta absoluta* and *Geotrichum candidum*.

Keywords: Tomato, *Tuta absoluta*, Survey, *Geotrichum candidum*, Essential oils, insecticidal and antifungal activities.

RESUME

Le rendement de la tomate (*Solanum lycopersicum* L.) a récemment chuté au Cameroun, ne pouvant pas de ce fait satisfaire la demande nationale et celle des pays voisins. De sévères infestations d'insectes, telle que *Tuta absoluta*, ont favorisé la pourriture de la tomate causée par *Geotrichum candidum*, qui a été l'une des principales raisons de cette baisse de productivité. La méthode la plus courante de gestion des dommages est l'application de pesticides chimiques de synthèse, qui sont bien connus pour leurs effets nocifs sur l'homme et l'environnement. Par conséquent, des stratégies alternatives basées sur l'utilisation de produits naturels pour augmenter le rendement de la production sont nécessaires. Les huiles essentielles, l'une des sources de pesticides biologiques les plus fiables, se sont révélées prometteuses en termes d'efficacité, de durabilité et de compatibilité avec d'autres mesures de contrôle dans le contexte des programmes de gestion intégrée. Le but de cette étude a été d'enquêter sur *Tuta absoluta* à Foumbot, le principal bassin de production de la tomate au Cameroon, et de déterminer les activités insecticides et antifongiques des huiles essentielles de *Thymus vulgaris* et de *Cymbopogon citratus*. Un questionnaire et un échantillonnage sur le terrain ont été utilisés pour mener l'enquête. Des entretiens ont été menés avec 56 producteurs de tomate de cinq villages, et la quantification des dommages a été réalisé dans 19 champs de tomate à production commerciale et répartis dans l'arrondissement de Foumbot. L'incidence et la sévérité de l'infestation par *Tuta absoluta* ont été évaluées par la technique des quadrats suivant un plan en « W » inscrit le long de chaque parcelle et l'échelle de sévérité de 1 à 5 respectivement. De plus, certains facteurs liés aux indices de dommages ont été évalués. L'hydrodistillation a été utilisée pour extraire les huiles essentielles de *Thymus vulgaris* et de *Cymbopogon citratus*, et la composition chimique a été évaluée à l'aide de la chromatographie en phase gazeuse couplée à la spectrométrie de masse. Le potentiel insecticide des huiles essentielles a été déterminée en evaluant leur toxicité par contact et par fumigation contre les larves de *Tuta absoluta*. *Geotrichum candidum* a été isolé à partir des fruits de tomate perforés par *Tuta absoluta* puis infectés et pourris. La morphologie et les traits moléculaires ont été utilisés pour l'identifier. Les huiles essentielles ont été testées pour leur efficacité antifongique sur la croissance mycélienne et la germination des conidies des pathogènes par dilution en milieu solide et microdilution en milieu liquide, respectivement. *Tuta absoluta* a infesté tous les champs échantillonnés dans differents villages avec une incidence et sévérité moyennes de 93.20% et 4.40 sur une échelle de sévérité de 1 à 5, respectivement. Les dommages ont été associés à la variété et au stade de croissance. Les valeurs les plus élevées

ont été enregistrées avec la variété hybride (incidence: 88,26%, sévérité: 4,46) et au stade de maturation des fruits (incidence: 97,61%, sévérité: 4,90). L'analyse de la composition chimique a révélé que l'huile essentielle de *Thymus vulgaris* était majoritairement constituée du Thymol (21,53%) et d'α-Pinène (Dextro) (17,43%), tandis que le Neral (34,48%) et le Géranial (34,37%) ont été majoritaires dans l'huile essentielle de la citronnelle. L'analyse statistique a indiqué que les deux huiles essentielles ont exercé de manière similaire des effets *knockdown* et insecticide par contact direct et par fumigation. Les paramètres biologiques résultants pour les huiles essentielles de la citronnelle et du thym ont été des valeurs de KD_{50} de 0,19 µL/mL et 0,59 µL/mL et LD_{50} de 0,33 µL/mL et 0,61 µL/mL respectivement pour la toxicité par contact et KD_{50} de 1,29 µL/mL et 2,57 µL/mL et LD_{50} de 1,48 µL/mL et 3,05 µL/mL pour la citronnelle et le thym respectivement pour la toxicité par fumigation. Les huiles essentielles ont exercé l'activité larvicide à travers l'effet anti-acétylcholinesterase avec des valeurs similaires de CI_{50s} de 2,57 et 3,06 µL/mL pour les huiles essentielles du thym et de la citronnelle respectivement. Le séquençage de la region ITS1-5.8S ADNr-ITS4 du gene et l'analyse phylogénique ont confirmé l'identité des champignons pathogènes comme étant des isolats de *Geotrichum candidum* (*Geotrichum candidum* G1, *Geotrichum candidum* G2 et *Geotrichum candidum* G3). Le test d'activité antifongique a montré que les valeurs de Concentrations Minimales Inhibitrices des huiles essentielles de *Cymbopogon citratus* et de *Thymus vulgaris* ont été respectivement 0,71 µL/mL et 5,67 µL/mL sur la croissance mycélienne de tous les pathogènes. L'inhibition de la germination des conidies a révélé que les Concentrations Minimales Inhibitrices ont été indeterminées pour l'huile essentielle de *Thymus vulgaris* et de 0,71 µL/mL; 1,42 µL/mL et 5,67 µL/mL pour l'huile essentielle de *Cymbopogon citratus* contre *Geotrichum candidum* G1, *Geotrichum candidum* G2 et *Geotrichum candidum* G3 respectivement. Les résultats obtenus démontrent que l'huile essentielle de *Cymbopogon citratus* pourrait être utilisée dans le contrôle de *Tuta absoluta* et *Geotrichum candidum*.

Mots clés: Tomate, *Tuta absoluta*, Enquête, *Geotrichum candidum*, Huiles essentielles, activités insecticide et antifongique.

INTRODUCTION

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a worldwide grown vegetable with substantial economic value (**Wakil *et al.*, 2018**). It is one of the major marketed gardening crop contributing to the livelihood of million families (**Temple, 2001**) and the world's most highly consumed vegetable due to the status of its fruits as a basic ingredient in a large variety of raw, processed or cooked foods (**OECD, 2017**). In Cameroon, tomato is the most cultivated vegetable and the second most produced fruits after sweet banana (**MINADER, 2012**). Tomato fruits are indeed rich in water, vitamins A, C, E, minerals and antioxydant compounds (**OECD, 2017**).

Tomato is grown in the world either in natural farms or in controlled environment. China and India are the leading world tomato producers. Cameroon ranks 19th producer in the world and 6th in Africa behind Egypt, Nigeria, Algeria, Morocco and Tunisia (**FAOSTAT, 2021a)**. Tomato is cultivated in all agroecologies zones of Cameroon with relatively high productivity (**MINADER, 2015)**. Recent data reveals that a yield of 128623 kg/ha of tamato was produced in 2017 compared to 119799 kg/ha in 2019. This clearly indicates 8824 kg/ha (1.07 fold) yield reduction (**FAOSTAT, 2021b**). Attempts to diagnose the causes of yield shortage points out pathogens and insects (**Fontem *et al.*, 1998; konje *et al.*, 2019).** The pests causing significant damages include Diptera (*Dacus cucurbitae* and *Dacus punctatifrons*), Hemiptera (*Aphis craccivora; Bemisia tabaci*; *Macrosiphum euphorbiae* and *Myzus persicea*), Lepidoptera (*Helicoverpa amigera, Spodoptera littoralis* and more recently *Tuta absoluta*) (**Fontem *et al.*, 1998; Djiéto-Lordon and Aléné, 2006; Heumou *et al.*, 2015; Konje *et al.*, 2019**).

Since its outbreak around 2010s, *Tuta absoluta* (Meyrick 1917) had rapidly spreaded in the main Cameroonian tomato production basins with substantial yield declines. In fact, *T. absoluta* (Lepidoptera: Gelechiidae) is an holometabolous insect, comprising four developmental stages: egg, larvae, pupae and adult (**Desneux *et al.*, 2010**). The larval stage is known to be the damaging as they feed and grows on the aerial organs of tomato plant with leaves and fruits being the most affected organs (**Caffarini *et al.*, 1999; Desneux *et al.*, 2010, Hernández-Fernández *et al.*, 2010**). On leaves, larvae feed on the mesophyll tissues where chloroplast organelles are found, decreasing photosynthetic potential, this lead to chlorosis and necrosis (**Rwomushana *et al.*, 2019**). Infested fruits

present galleries of larvae and are characterized by holes which constitute penetration routes for pathogens fungi such as *G. candidum*. In addition, *G. candidum* infections produce mycotoxins that affects the organoleptic, nutritional and market values of the fruits. In many production basins across the world, 90 to 100% yield losses in both fruits quality and quantity have been ascribed to *T. absoluta* (**Estay, 2000; EPPO, 2005; Ayalew, 2015**). Up to date, to the best of our knowledge, no study has reported the extend of losses related to *T. absoluta* in Cameroon, although, good agricultural pest monitoring is critical as it offers pre-requisites towards planning and implementing a proper control measures.

To date, despite its outbreak in some commercial fields in the western highlands, the pest has not received any attention (**Konje *et al.*, 2019**). Farmer's has been desperately using a set of synthetic products like Emamectin benzoate, Lynx and Propiconazole to come across both the *T. absoluta* and *G. candidum*. Whereas, drawbacks as build up of pesticide residues on tomato fruits cause human toxicity and environmental pollution. Accordingly, the Environmental Protection Agency (EPA) suggests the promotion of natural alternative, based on their eco-safety and the cost effectiveness. Plant-based control strategy exhibiting insecticidal and antifungal activities have become extremely important, since plants have physiological properties or defense mechanisms, through active metabolites that protect against insects and prevent pathogens invasion (**Bennett and Wallsgrove, 1994)**. Thus, alternative approaches to control *T. absoluta* and *G. candidum* include the utilization of plant-derived active metabolites with insecticidal and antifungal properties (**Stappen *et al.*, 2018; Debbabi *et al.*, 2020; Ghasemi *et al.*, 2020**). Then, the repellent (**Krcmar and Gvozdic 2016**), fumigant (**Campolo *et al.*, 2014**), antifeedant (**Hernández-Lambraño *et al.*, 2014**), contact (**Slimane *et al.*, 2014**) and antifungal (**Plotto *et al.*, 2003; Liu *et al.*, 2009; Nana *et al.*, 2015**) activities of botanical extracts including essential oils are well documented (**Ferrari de Brito *et al.*, 2015**; **Samira and Habib, 2017**). The latters are sustainable and compatible with other control measures in the framework of Integrated Pest Management (IPM) programs (**Regnault-Roger, 1997**). Essential oils from *Thymus vulgaris* L. (Lamiaceae) and *Cymbopogon citratus* (DC.) Stapf. (Poaceae) plants used in Cameroon for culinary purposes, have been shown to have insecticidal and antifungal properties on a wide range of arthropod pests and fungi (**Liu *et al.*, 2009; Costa *et al.*, 2013; Tak *et al.*, 2015; Tančinová *et al.*, 2021**) of many crops including tomato. Essential oils exert insecticidal effect mainly through neurotoxic action

or by inhibition of the activity of detoxifying enzymes (**Potter and Wadkins, 2006; Mohamed *et al.*, 2016**). Preliminary works suggested that acetylcholinesterase (AChE) found in the nervous system might be the main target of the essential oil, and the decline of the AChE enzyme activity could alter the functioning of the nervous system causing the death of the insects (**Liao *et al.*, 2017)**.

General research question

May essential oils from *T. vulgaris* and *C. citratus* contribute to control *T. absoluta* and *G. candidum* damaging tomato in Foumbot area?

Specific research questions

Specifically:

1. What is the level of damages associated to *T. absoluta* in Foumbot area?
2. Essential oils from *T. vulgaris* and *C. citratus* could be used to fight against *T. absoluta*?
3. Essential oils from *T. vulgaris* and *C. citratus* could be used to control *G. candidum*?

General hypothesis

Hence, we hypothesised that, essential oils from *T. vulgaris* and *C. citratus* could be used in the control of *T. absoluta* and *G. candidum* associated to tomato rot in Foumbot.

Specific hypotheses

1. *Tuta absoluta* causes significant damages in Foumbot subdivision;
2. Essential oils from *T. vulgaris* and *C. citratus* have insecticidal activity against *T. absoluta*;
3. Essential oils from *T. vulgaris* and *C. citratus* have antifungal effect against *G. candidum.*

General objective

The overall goal was to determine the insecticidal and antifungal activities of *T. vulgaris* and *C. citratus* essential oils against *T. absoluta* and *G. candidum* associated to tomato rot in Foumbot production basin.

Specific objectives

More specifically, this study aimed to:

1. Survey on *T. absoluta* in Foumbot production basin;
2. Determine the insecticidal activity of *T. vulgaris* and *C. citratus* essential oils against *T. absoluta;*
3. Assess the antifungal activity of *T. vulgaris* and *C. citratus* essential oils against *G. candidum* associated to *T. absoluta* infestation.

LITERATURE REVIEW

CHAPTER I: LITERATURE REVIEW

I.1 The host plant (*Solanum lycopersicum* L.)

I.1.1 Origin and distribution

The natural origin of *Solanum lycopersicum* (*S. lycopersicum*) has been localised in the narrow band between the Andes mountain ranges and the Pacific coast of western South America. This extends from southern Ecuador to northern Chile, including the Galapagos Islands. This is based on the geographic distribution of the native wild ancestors of cultivated tomato located between coordinates 0º-20º S and 64º-81º W where they grow spontaneously (**OECD, 2017**). The three wild species most closely related to cultivated tomato include the red-fruited specie *S. pimpinellifolium*, the orange-fruited species, *S. galapagense* and *S. cheesmaniae* (**Menda *et al.*, 2013**). The characteristics of these wild fruits were small size (1-2 cm diameter), bilocular and acid taste (**OECD, 2017**). Tomato was brought to Europe by the Spanish conquistadors in the 16th century and later introduced from Europe to southern and eastern Asia and Africa (**Naika *et al.*, 2005**). The first appearance of the tomato in Africa may have been in a "*jardin de naturalisation*" created and run by a French government in Senegal from 1816 to 1827 (**Alpern, 1992**). The first improved tomatoes were developed by Italian breeders in the early 18th century, who converted the small, wrinkled and hard tomato into the red coloured, smooth and juicy varieties known today. Starting from these cultivars, the United States begun in 1867 the production of various cultivars and nine commercial varieties (Early smooth, Cook's favorite, Tildem, Powells early, Feue, Large red, Large yellow, Tree tomato red and Yellow plum) (**OECD, 2017)**. Thus, tomato is now a cosmopolitan crop.

I.1.2 Taxonomy

The common name known all over the world, tomato is assigned to the Mexican word in Náhuatl "*xictomatl*" ("*xictli*": navel and "*tomatl*": tomato), meaning the tomato with a navel (scar left on the fruit by the peduncle). The genus *Solanum* consists of approximately 1500–2000 species including *Solanum lycopersicum* Linnaei (1753) (**Darwin *et al.*, 2003**).

Taxonomic classification (**Linnaei, 1753**)

Kingdom : Plantae

Division : Angiosperm

Class : Mangoliopsida

Order : Solanales

Family : Solanaceae

Genus : *Solanum*

Specie : *Solanum lycopersicum*

Synonym : *Lycopersicon esculentum* Miller (1768)

I.1.3 Botanical description

Tomato is an annual herbaceous plant cultivated in tropical and temperate climates (**Blanca *et al*., 2012; OECD, 2017**). The five growth stages of plants are described by **García *et al.* (2011)** and **Jones (2013)** as followed (Figure 1):

- Germination and early growth from sowing to initial leaves (between 25 and 30 days);
- Vegetative period from initial leaves appearance to early flowering (20 to 25 days);
- Fowering period from first flower buds to first fruit appearance (20 to 30 days);
- Early fruiting from first fruits to early maturity (20 to 30 days);
- Fruit maturity (15 to 20 days).

The duration of these stages depend on the varieties and environmental factors such as air temperature, light condition, soil conditions and nutrients availability (**Shamshiri *et al*., 2018**). The average duration from seeding to harvesting of the first fruits varies from 45 days to over 100 days, depending on the maturity level of the cultivar (**Jones, 2013**). It should be noted that tomatoes are harvested two to four times per week, and only when they have reached the mature green stage (vine-ripe), as they start to ripen.

Tomato plant may reach up to 3 m in height. The tomato root system grows to a depth of 50 cm or more. The main root produces dense lateral and adventitial roots. The stem growth habit ranges between erect and prostrate and grows to height of 2-4 m. It is covered by hairy and glandular trichomes that confer a characteristic smell. Leaves are alternately

arranged on the stem with a 137.5° phyllotaxy. The leaves, spirally arranged around the stem are covered by glandular, hairy trichomes and are simple or compound. Compound leaves are typically made of five to nine leaflets. Leaflets are petiolated and dentated. The flowers are yellow, 1-2 cm long and 1.5-2 cm diameter, with five pointed lobes on the corolla bisexual. Flowers are bisexual, growing opposite or between leaves. Calyx tube is short and hairy, sepals are persistent. Ovary have 2-9 compartments. The fruits are classified botanically as berries with globular or ovoid form, their size vary from 15 g to more than 50 g. The immature fruit is green, ripe fruits colour ranged from yellow, orange to red. The tomato seeds are numerous, kidney shaped with 3 to 5 mm wide or pear shaped with 2 to 4 mm wide. The embryo is coiled up in endosperm and is covered by a strong seed coat called the testa (**OECD, 2017**).

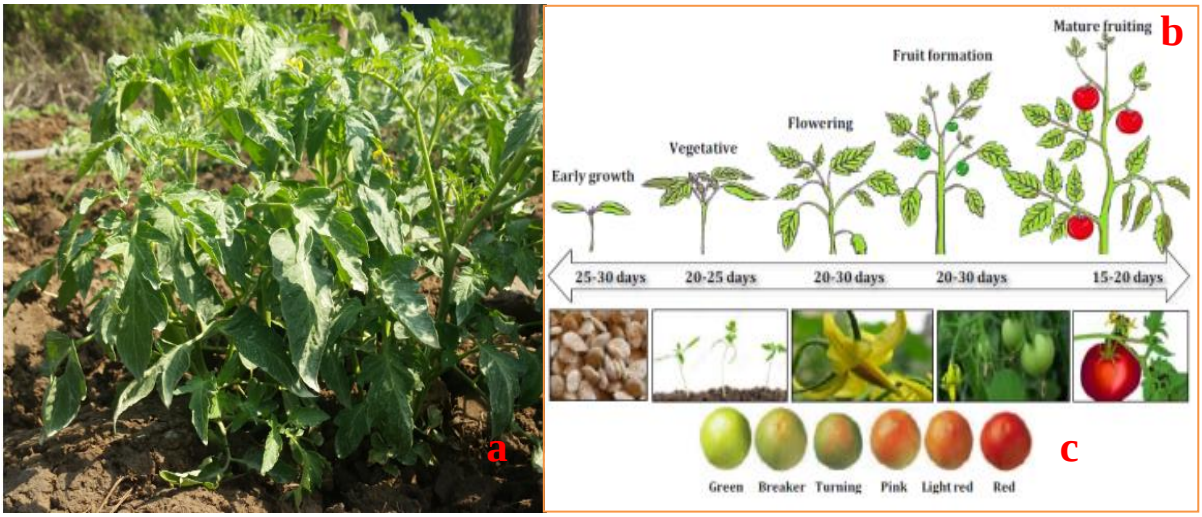

Figure 1: Tomato plant (a) and tomato growth stages (b) with different levels of fruit ripeness (c)

I.1.4 Ecology of Tomato

Tomato cultures require a warm climate and abundant sunshine for best growth and development. It does not tolerate frost. Its optimum temperature is around 26ºC during day and 12ºC at night. Air relative humidity between 55-60% is important for effective pollen production and pollination. Water stress and long dry period cause flowers dropping and fruit splitting. Tomatoes better develops deep, well-drained sandy loam soil and grow well on most mineral soils. Yield tomato production is higher when pH of soil is between 6.5 and 6.9 compared with that obtained in acidic soils. Soils with acidic pH or salinity lead to a decrease in the size of the fruit (**OECD, 2017**).

I.1.5 Importance of Tomato

I.1.5.1 Economic importance of tomato

Tomato is the world's most consumed vegetable (**OECD, 2017)**. It is Africa's most consumed fruit or vegetable and also one of the most researched of all horticultural crops (**Ajibade *et al.*, 2017**). In Cameroon, tomato is the most widely cultivated vegetable. Moreover, it is one of the major marketed gardening crop and rank second among produced fruits after sweet banana (**MINADER, 2012; Heumou *et al.*, 2015**). In addition to its consumption as a raw vegetable or being added to other food items, tomato may be processed into a variety of foods of high economic importance, including concentrate and sauce.

I.1.5.2 Nutritional importance of tomato

The fruit contains a large quantity of water, vitamins, minerals and fibers, low amounts of proteins, fats and some carbohydrates. It also contains carotenes, such as lycopene (which gives the fruit its predominantly red colour) and beta-Carotene (which gives the fruit its orange colour) (Table 1) (**OECD, 2017**).

Table 1: Nutritional composition of tomato

Content	/100g of Red Tomato
Energy	18 K cal
Carbohydrate	3.9 g
Fat	0.2 g
Protein	0.9 g
Water	94.5 g
Vitamin A (β-carotene)	833 mg
Vitamin B1(Thiamin)	0.037 mg
Vitamin B3 (Niacin)	0.594 mg
Vitamin B6 (pyridoxine)	0.08 mg
Vitamin C (acide ascorbique)	14 mg
Vitamin E (tocopherol)	0.54 mg
Vitamin K (phylloquinone)	0.0079 mg
Mg	11 mg
Mn	0.114 mg
Fe	0.3 mg
Cu	0.19 mg
S	24 mg
Cl	38 mg
Na	5 mg
Ca	20 mg
P	24 mg
K	237 mg
Lycopene	2.537 mg
Oxalic acid	2 mg

Mg : Magnesium, Mn :Manganese, Fe : Iron, Cu : Copper, S :Sulphur, Cl : Chlorine, Na : Sodium, Ca :Calcium, P :Phosphorus and K :Potassium

I.1.5.3 Health benefits

Tomatoes fruits are valuable vegetables considering their nutritional values. As fruits are free of cholesterol, dieticians and nutritionists recommend tomatoes in cholesterol controlling and weight reduction diet-programs (**Ntonifor *et al.*, 2013**). Epidemiological and clinical studies have shown that increasing consumption of tomatoes was able to prevent the occurrence of certain diseases as cancer and cataract. This is due to the presence of large

amount of phytochemical compounds including families of carotenoids and flavonoids (**Nassarawa and Sulaiman, 2019**). These compounds have been reported to play important roles of antioxydants (**Eldahshan and Singab, 2013**). Antioxidants are specific compounds that protect human, animal and plant cells from the damages effects of free radicals (**Tiwari and Husain, 2017**). Lycopene, the major antioxidant with approximatively 90% of the total carotenoid contents, is 1.16 folds higher than β-carotene and 2.9 folds higher than L-ascorbic acid (**Nassarawa and Sulaiman, 2019)**. Lycopene act by neutralizing dangerous free radicals that otherwise damage cells and cell membranes. Then, lycopene has proven to be protective and reduce severity of many types of cancers as prostate, colon, cervical and rectal's cancers (**Kucuk, 2001; Kumar *et al*., 2012).** β-carotene prevent lung's cancer by protecting phagocytic cells from oxidative damage occuring during this affection**.** Moreover, β-carotene improving vision and may help to protect the eyes from the damage that can lead to cataracts **(Nassarawa and Sulaiman, 2019)**. Tomato also contains flavonoids as chalconaringenin which is the predominant, representing 35 to 71% of the total flavonoid (**Slimestad *et al*., 2008**). Chalconaringenin action include suppression of reactives oxygenated species formation by inhibition of enzymes, scavenging free radicals, and regulation of antioxidant defenses (**Mishra *et al*., 2013**).

I.1.6 Agronomic practices

Agronomic practices are activities performed before and during tomato plants cultivation. Its include raising of nursery, land preparation, transplantation, fertilization, irrigation, pruning of plants, Earthing-up and harvest.

I.1.6.1 Raising of nursery

Tomato seeds are sown on smooth and well leveled raised beds of 3 x 0.6 m size and 10-15 cm in height. Raised beds are necessary to avoid problem of water logging in heavy soils. In sandy soils, sowing are taken up in flat beds. Draw lines should be 10-15 cm apart. Prior to sowing, well decomposed organic matter is added. Sowing should be done thinly in lines spaced at 10-15 cm distance with a depth of 2-3 cm. Seedbeds are wattered twice a day to ensure sufficient moisture for germination. The seedlings of 15-25 cm tall with 3-5 true leaves are ready for transplantation within 4-5 weeks of sowing (**Naika *et al*., 2005**).

I.1.6.2 Land preparation

Ploughing (or digging) is necessary to prepare the land for a new crop. It improves the structure and water holding capacity. The field land which will receive the seedlings must be

humid. Nutrient requirements of the tomato crop depend to factors including variety and yield expected (**OECD, 2017**).

I.1.6.3 Transplantation

During removing the seedlings from the seedbed, a large clump of soil is keep attached to the roots to prevent them from damages. Plants is watered immediately once they have been transplanted around the base of the plant to settle the soil. Spacing between plants and rows depends on the cultivar growth habit, soil type, cropping system and also whether the plants will be supported by stakes or left on the ground. Then, the common spacing varies from 25-50 cm between seedlings and 1.50-1.80 m between rows (**OECD, 2017; Naika *et al*., 2005**).

I.1.6.4 Fertilization

To optimize yields, tomatoes need to be fertilised. In general, fertiliser is applied during three stages: first, before transplantation; second, 60 days afterwards; and third, after 100 days (**OECD, 2017**). There are two groups of crop nutrients: organic manures and chemical fertilisers (**Naika *et al*., 2005)**.

There are three types of organic manures know as farmyard manure, poultry manure and compost. The mean level is 30 tons per hectare (t/ha). The most common kinds of farmyard manures are horse, cow and pig manures. Of these three kinds, horse manure has the best balance of nutrients. Poultry manure is chicken manure and plants absorb easily the nutrients from it. Compost derived from organic materials as crop residues, garden cuttings and kitchen wastes (**OECD, 2017; Naika *et al*., 2005**).

Chemical fertilizers enriche the soil by adding nutrients. It is subdivided into two groups, compound and simple fertilisers. Compound fertiliser is a mixture of nitrogen (N), phosphorus (P) and potassium (K) at mean level of 50 kilograms per hectare (kg/ha) for nitrogen (N), 90 kg/ha for phosphorus (P) and 225 kg/ha for potassium (K) (**OECD, 2017**). Simple fertilisers as sodium nitrate and urea are used when a crop has a specific deficiency.

I.1.6.5 Irrigation

Poor irrigation conditions can cause radial and concentric cracking on the fruit. This is a serious physiological disorder that affects tomatoes unmarketable and leads to quick deterioration. Moisture requirements vary with crop variety, prevailing climate and soil characteristics. Several irrigation methods have been reported as surface, sprinkler and drip

irrigations (**Naika *et al*., 2005)**. Drip irrigation is commonly used and involves dripping water onto the soil at very low rates (2-20 litres/hour) from a system of small diameter plastic pipes fitted with outlets called emitters or drippers. Water is applied close to plants so that only part of the soil in which the roots grow is wetted, unlike surface and sprinkler irrigation, which involves wetting the whole soil profile. Drip irrigation provides a very favourable high moisture level in the soil in which plants can flourish and also allows the application of fertilisers mixed in the irrigation water (**OECD, 2017**).

I.1.6.6 Pruning of plants

The need for pruning depends on the type of plant (indeterminate type, semi-indeterminate type and determinate type). Indeterminate type and semi-indeterminate type need to be prune as plant continue to grow after flowering. If plants are not pruned, they will grow at random and fruit will be smaller. Pruning improves the light penetration and air circulation, stimulates plant development and more efficient phytosanitary control, then achieve higher quantitative and qualitative yield. Plants can be supported by a trellis of 2-metres posts (sunk to 50 cm) positioned at regular intervals of 3-5 metres (**OECD, 2017**).

I.1.6.7 Earthing-up

Earthing-up consists of massing up earth at the plant base with the aim to assure the growth of adventitious roots providing better anchorage. The first earthing-up occurs between the first and second week after transplantation and is repeated between the fourth and fifth week. On occasion, this practice is also performed during weeding (**OECD, 2017**).

I.1.6.8 Harvest

The level of maturity at which fruits are harvested depends on the final production goal. Thus, for local consumption, harvested fruits should have intense red and pink colour. The harvest interval may continue up to seven months. Processed fruits should have physiologic maturity at the harvest and exported tomato are “Green-mature” (**OECD, 2017**).

I.1.7 Tomato production

Tomato is grown worldwide for domestic use or as commercial (local, regional, national or international) purpose. In 2017, the world production was about 171 million tons from about 5 million hectares. In Africa, total production amounts to 37.8 million tons annually with Cameroonian production estimated at 1.279.853 tons for yield level of 12.1243Kg/ha (**FAOSTAT, 2017**). This value is much lower compared to the worldwide average yield (37.6004 Kg/ha) (**FAOSTAT, 2017**). The major tomato-producing countries are

China and India (**FAOSTAT, 2021**). Cameroon is 19th producer in the world and ranked 6th in Africa behind Egypt, Nigeria, Algeria, Morocco and Tunisia (**FAOSTAT, 2021**).

I.1.8 Tomato production constraints

Tomato production is constrained by numerous abiotic and biotic factors.

I.1.8.1 Abiotic constraints

The major abiotic constraints for plant growth, development and production is waterlogging. In fact, waterlogging affect soil properties and development of plant organs.

Through soil properties, physical, chemical, electro-chemical and biological changes are noted. As physical variation, soil compaction, bulk density and carbon dioxyde (CO_2) increase contrary to the diffusion of dioxygen gase (O_2) which decreases. As long as the level of oxygen available in soil is reduced, anaerobic microorganisms dominate, creating effects in the rhizosphere in which Fe^{2+}, Mn^{2+}, H_2S, lactic acid, and butyric acid, increase to toxic concentrations which are potentially damaging to plant roots. Electro-chemical change consists of pH decreasing and is cause by the accumulation of volatile organic acids as well as the high concentration of CO_2 which reduces root growth. Waterlogging impedes the ability of soil to provide an optimum medium for plant growth and alters its physical, chemical and biological characteristics. Fundamentally, the soil should have optimal water and air content for the proper physiological performance of all phases of plant growth. All of these lead to restricted root growth, reduced tiller number, premature leaf senescence and production of sterile florets thus affecting the poduction yield of up to 50 % in severe cases (**Manik *et al.*, 2019**).

Waterlogging inibit development of plant organs as leaves, stem and root. One of the first plant responses to waterlogging is the reduction in stomata conductance. The stomata closure was attributed to abscisic acid (ABA) transport from older to younger leaves or *de novo* synthesis of this hormone. The closure of stomata alters both gaseous exchange and process of transpiration, and usually results to wilting of plant leaves, senescence earlier than usual as well as foliar abscission (**Ashraf, 2012**). Waterlogging also decreases the leaf chlorophyll content, then reduce the photosynthetic capacity of plants (**Manik *et al.*, 2019**).

I.1.8.2 Biotics constraints

In Cameroon and elsewhere, insect pests and pathogens are the most important biological limitations to tomato production (**Fontem *et al.*, 1998; Lakshmi *et al.*, 2019**). In Cameroon,

the insect pests that hinder tomato production include Diptera (*Dacus cucurbitae* and *Dacus punctatifrons*), Lepidoptera (*Helicoverpa amigera* and *Spodoptera littoralis*), Hemiptera (*Aphis craccivora*; Bemisia tabaci; *Macrosiphum euphorbiae* and *Myzus persicea*), and more recently Lepidoptera (*Tuta absoluta*) (**Fontem *et al*., 1998; Djieto *et al*., 2006; Heumou *et al*., 2015; Konje *et al*., 2019**).

I.2 *Tuta absoluta*: A key pest of tomato

I.2.1 Taxonomy and host range

I.2.1.1 Taxonomy of *Tuta absoluta*

Tuta absoluta was originally described as *Phthorimaea absoluta* by Meyrick in 1917 from a single male which was collected in Huancayo, Peru. The arrangement of the genus of this specie has been changed three times and was subsequently classified in different genera as *Gnorimoschema* by Clarke in 1962, *Scrobipalpula* by Povolny in 1964 and *Scrobipalpuloides* by Povolny in 1987 until it was redescribed under the genus *Tuta* as *Tuta absoluta* (**Povolny, 1994**).

Taxonomic Classifcation of *Tuta absoluta* (Povolny, 1994)

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Arthropods
Class: Insects
Order: Lepidoptera
Family: Gelechiidae
Genus: Tuta
Specie: *T. absoluta*

Common names: Tomato leafminer, South American tomato moth, Tomato borer

Synonyms: *Phthorimaea absoluta* Meyrick (1917), *Gnorimoschema absoluta* Clarke (1962), *Scrobipalpula absoluta* Povolny (1964), *Scrobipalpuloides absoluta* Povolny (1987)

I.2.1.2 Host range of *Tuta absoluta*

Although the main host of *T. absoluta* is tomato (*Solanum lycopersicum* L.), several Solanaceae both cultivated and wild species may host *T. absoluta*. The cultivated host species include potato (*Solanum tuberosum* L.), aubergine (*Solanumm elongena* L.), and pepper

(*Solanum muricatum* L.), while Black nightshade (*Solanum nigrum* L.) and thorn apple (*Datura stramonium* L.), are among its wild hosts (**Ayalew, 2015**).

I.2.2 Origin, pathways of entry and establishment of *Tuta absoluta*

The South American tomato moth, *T. absoluta,* has been a key pest of tomato in South America since the 1950s and established itself in countries of Latin America such as Argentina, Bolivia, Brazil, Colombia, Ecuador, Panama, Paraguay, Peru, Uruguay, Venezuela and Chile (**Biondi *et al.,* 2018**). Chile is the point of introduction of *T. absoluta* in Europe through Spain in 2006 (**Desneux *et al.,* 2011**; **Guillemaud *et al.,* 2015**). From this last country, it has spread to different parts of the world including Africa (**Brévault *et al.,* 2014**; **Rwomushana *et al.,* 2019**). In the Africa, *T. absoluta* was first reported in Algeria, Morocco and Tunisia in 2007. Thus, has invaded 41 of the 54 African countries including Cameroon (**Mansour *et al.,* 2018; Konje *et al.,* 2019; Rwomushana *et al.,* 2019**). Different pathways are involved in facilitating the entry and spread of *T. absoluta* in the invaded countries. Fruits importation and packaging materials from infested countries have been cited as the main infestation sources in several countries over long distances (**Caceres, 1992**; **Desneux *et al.,* 2010**; **EPPO, 2010**; **Karadjova *et al.,* 2013**). Therefore, wind-assisted natural spread of the adult is an important factor in the local distribution of *T. absoluta* (**Desneux *et al.,* 2010**; **Rwomushana *et al.,* 2019**).

I.2.3 Biology and Ecology of *T. absoluta*

Females of *T. absoluta* use plant volatiles attractants such as β-phellandrene, limonene, 2-carene, and (E)-β-caryophyllene for orientation toward host plants (**Proffit *et al.,* 2011**). On tomato leaves, Adult females release sex pheromones to attract males and exhibit mating behaviours from a few minutes up to six hours (**Vacas *et al.,* 2011**; **Lee *et al.,* 2014**). The main compounds of sex pheromones are tetradecatrienyl acetate and tetradecadienyl acetate in the proportions of 91:9, respectively. Females mate only once a day and are able to mate up to six times during their lifespan varying from 10 and 15 days for female and 6-7 days for males (**Estay, 2000**). Duration of a single mating is 4–5 hours (**Desneux *et* al., 2010**). Oviposition begins within 1 to 2 days after mating. During 7days, females lay 76% of their eggs. Female insect lay between 250 to 300 eggs in its life time. At temperatures between 25 to 30°C, the eggs hatch in 6 to 7 days into larva. The optimal temperature for *T. absoluta* development is 30˚C, and life cycle duration varies according to temperature with average development time of 76.3 days at 14 ºC, 39.8 days at 19.7 ºC and 23.8 days at 27.1 ºC (**Barrientos *et al.,* 1998**). Although no development or reproduction occurs at low

temperature, *T. absoluta* shows cold tolerance, with 50% larval, pupal, and adult survival at 0˚C (**Cuthbertson *et al.*, 2013, Biondi *et al.*, 2018**). These biological characteristics enable *T. absoluta* to undergo up to 10-12 generations per year and to survive during cold seasons in protected and open-field crops.

I.2.4 *Tuta absoluta* life cycle and description

Tuta absoluta life cycle comprises four development stages: egg, larva, pupae and adult (Figure 2). Eggs are Small, 0.36 mm long and 0.22 mm large, cylindrical, creamy white to yellow. Eggs are mainly deposited individually and preferentially on the underside of leaves. Hatching takes place after 4–5 days after oviposition (**EPPO, 2005**; **Cocco *et al.*, 2015**). The Larvae is Creamy, light yellow or whitish after eclosion with dark head, becoming greenish to light pink in the second to fourth instars according to food (leaflet or ripe fruit). First instar is 0.9 mm long and fourth is 7.5 mm long. The larval duration from first to fourth instar is 13 to15 days (**EPPO, 2005**). Mature larvae (3rd and 4th instar) usually drop to the soil where they produce a thin cocoon to transform into pupae, although pupation may also occur on the leaves. The pupae with have cylindrical form, greenish coloration at first time, turning chestnut brown and dark brown near to the adult emergence. The pupal duration is 9–11 days. Adult moths are about 10 mm long, silverfish-grey scales, filiform antennae, black spots on anterior wings (**EPPO, 2005**; **Ajibade *et al.*, 2017**). The female is wider and more voluminous than the males.

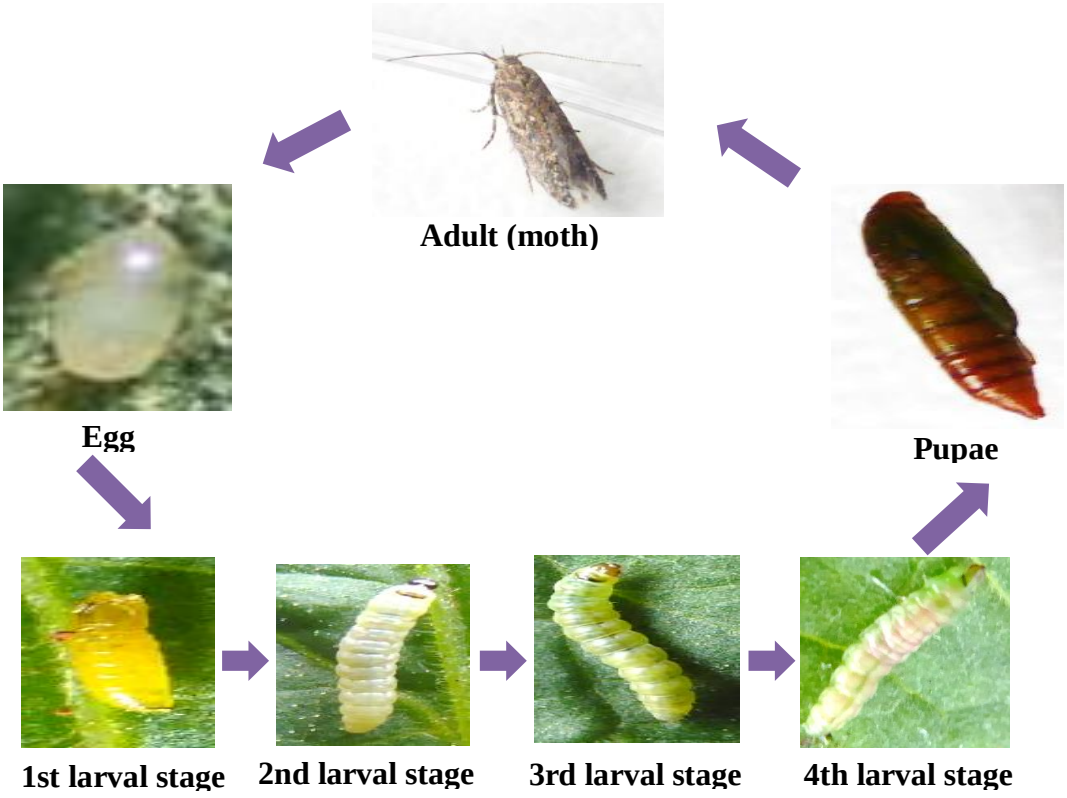

Figure 2: *Tuta absoluta* life cycle

I.2.5 *Tuta absoluta* damages

Tuta absoluta has been classified in 2020 priority pest list as pest of economic and environmental importance (**OPEP Impact Assessment Model, 2019**). Indeed, it is an Endophagous herbivorous insects that lives within plants (mainly on leaves, flowers and fruits) and leads to the formation of plant architecture mines. Tomato plants are infested at any developmental stage from germination to mature fruiting (**Braham and Hajji, 2012**). After hatching, the larvae, the damaging stage wandered around the leaf surface for an average of 12 minutes and approximately 15 mm from its egg shell before starting to graze on the leave surface (**Cuthbertson *et al.*, 2013**). Larvae infest aerial plant organs but leaves and fruits are mainly damaged (**Yankova and Ganeva, 2013)**. Flowers burning and dropping, stems present larvae mediated-mines, distortion and wilt (**Desneux *et al.*, 2010; Yankova and Ganeva, 2013**). Leaves infested by *T. absoluta* present abnormal form and folds. Feeding resulted in conspicuous larvae mediated-mines under leaves cuticles. In fact, the larvae penetrate the leaf and feed the mesophyll tissues locating chloroplast organelle and leaving the epidermis intact. Thus, the photosynthesis activity of the leaves is thereby reduced which result in chlorosis, early drying, burning apparence and necrosis (Figure 3) (**Ballal *et al.*, 2016**; **Rwomushana *et al.*, 2019**).

Figure 3: *Tuta absoluta* damages on tomato leaves in early (a) and late infestation (b)

Infested fruits present galleries of larvae and are characterized by reduce size, pinhead holes size which constitute routes for secondary infection by pathogens leading to fruit rot with production of toxins, and thereby directly reducing crop value (Figure 4) (**Moline, 1984; EPPO, 2005; Ekesi *et al.*, 2011).** Owing to its leaf-mining activity and through fruit infestation, *T. absoluta* causes drastic tomato yield losses of 100% (**EPPO, 2005; Ayalew, 2015**).

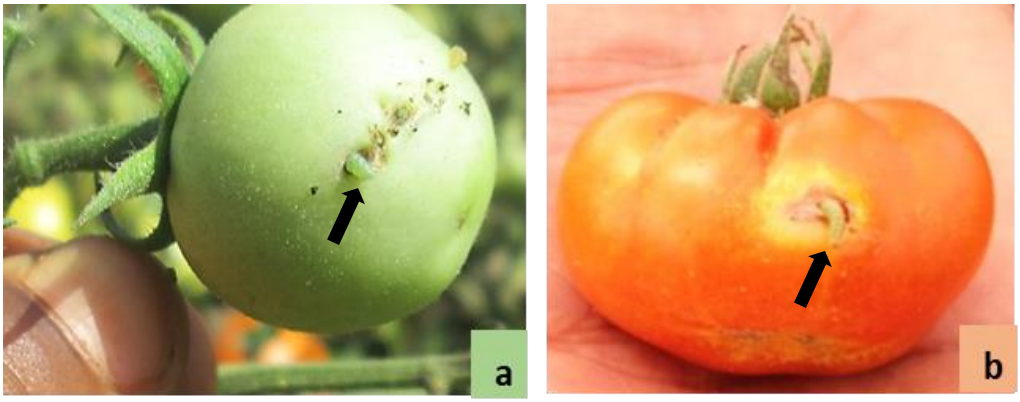

Figure 4: Wounds created by *Tuta absoluta* on unripe (a) and ripe (b) tomato fruits causing fungal infection

Among pathogens which hinder tomato production, fungi are the most predominant, causing severe diseases (**Fontem *et al.*, 1998)**. Moreover, phytopathogenic fungi causes alterations during different plant-stages growth, and in post-harvest stage (**Jiménez-reyes *et al.*, 2019**). Such pathogens increase damage by causing rot which renders fruits unfit to consumption due to the mycotoxins production (**Ekesi *et al.*, 2011**; **Van de Perre *et al.*, 2013**) which can generate poisonous disorders such as genotoxicity, nephrotoxicity, hepatotoxicity, and immunosuppression in consumers (**Lee *et al.*, 2007; Satish *et al.*, 2007; Díaz-Dellavalle *et al.*, 2011**). In addition, rot deteriorate organoleptic and nutritional values of fruits thus, lowering their market value (**EPPO, 2005)**. Such pathogens which limit tomato production include *Phytophthora infestans*, *Alternaria solani*, *Rhizopus stolonifer* and *Geotrichum candidum* (**Fontem *et al.*, 1998; Fiedler, 2014; Tančinová *et al.*, 2021)**. The last causes sour rot which is one of the major limiting factor of tomato production (**Fiedler, 2014**).

I.3 *Geotrichum candidum*

I.3.1 Taxonomy and host range

I.3.1.1 Taxonomy of *Geotrichum candidum*

The genus *Geotrichum* is considered as a filamentous fungus that resembles yeast and is called " yeast- like " fungi. This genus is composed of 18 species amongst which *Geotrichum candidum* Link (1809) classified as followed (**Kurtzman and Fell, 1998; Barnett *et al.*, 2000; Pottier *et al.*, 2008; De Hoog and Smith, 2004**):

Domain: Eukaryot

Kingdom: Fungi

Division: Ascomycota

Class : Hemiascomycetes

Order : Saccharomycetales

Family : Candidaceae

Genus : Geotrichum

Specie : *G. candidum*

Synonyms: *Endomyces geotrichum* Butler and Petersen (1972), *Galactomyces geotrichum* Redhead and Malloch (1977), *Dipodascus geotrichum* Antonie van Leeuwenhoek (1977)

I.3.1.2 Host range of *Geotrichum candidum*

Geotrichum candidum can cause epidermal and lung infections in immunocompromised patients. As a plant pathogen, *G. candidum* causes disease in many fruits and vegetables including tomato (*Solanum lycopersicum*), carrot (*Daucus carota* subsp. sativus), potato (*Solanum tuberosum*), stone fruits (*Prunus* spp), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*) (**Fiedler, 2014**).

I.3.2 Origin, pathways of entry of *Geotrichum candidum*

Geotrichum candidum is an ubiquitous pathogen considered as natural flora, it is a spoilage pathogen of foods, and a human pathogen. It can be found in all components of agriculture, including soil, water, and dust. Healthy tomato fruit are infected by *G. candidum* through microcracks and wounds which offers route to the infection (**Bartz *et al.*, 2009**). Then, *G. candidum* is a wound pathogen, requiring injury for entry. Moreover, it is a common saprophyte easily recovered for stagnant water and wet soil (**Moline, 1984**).

I.3.3 Characterization

I.3.3.1 Morphological characterization

The morphology of *Geotrichum* spp on Potato Dextrose Agar (PDA) show milky white colony, conodiophores differentiated into disarticulated hyphae then into rectangular cells called arthric conidia (**De Hoog *et al.*, 1986)**.

Geotrichum candidum is on the borderline between typical yeasts and moulds. It is charactarized by white to cream-coloured colony, expanding growth over 50 mm diameter in 10 days. Hyaline hyphae, with wide vary from 7 to 12 µm differentiated into disarticulated

hyphae, then into arthric conidia (**De Hoog *et al.*, 1986)**. Within species two main morphotypes have been described. One is characterised by strains with cream-coloured, yeast-like colonies that produce abundant arthroconidia and generally have only slight growth and proteolytic activity, an optimal growth temperature between 22 and 25 ^{o}C and an acidifying activity. The other type forms white felting colonies, with a predominance of vegetatif hyphae and few arthroconidia, and has high proteolytic activity, rapid growth at optimal temperature of 25–30 ^{o}C, and an alkalising activity. A third type can be defined, including those strains that fall between the two well-defined types (**Boutrou and Guéguen, 2005)**.

I.3.3.2 Molecular characterization

The genus *Geotrichum* cannot be identified by microscopic morphology alone because many related and unrelated fungi form arthrospores and have similar characteristics. Within *G. candidum* there is variable phenotypical characteristics and very marked polymorphisms, making methods ideal for identification and sub-group classification of *G. candidum* isolates (**Prillinger *et al.*, 1999**). DNA sequencing of the internal transcribed spacer (ITS) regions is a useful and accurate approach for identification of fungal organisms found in the food chain as Geotrichum genus (**De Hoog and Smith, 2004**; **Buehler *et al.*, 2017; Ma *et al.*, 2017**).

I.3.4 *Geotrichum candidum* damage

Geotrichum candidum is an opportunist fungi firstly observed infecting tomato fruit in 1923 by Prichard and Porte (**Fiedler, 2014**). Through a wound, *G. candidum* infect tomato fruit and causes sour rot at all steps of tomato production and during the postharvest setting. Symptoms of infection with *G. candidum* include brown water-soaked lesions that contains white mycelium. As decay progresses, fruit become soft and unmarketable. A distinctive sour odor is associated with the disease (**Moline, 1984**). Following damages due to *T. absoluta* and mediated by *G. candidum* infection, tomato growers used differents control measures.

I.4 Management of *Tuta absoluta* and *Geotrichum candidum*

Differents control measures are used to manage *T. absoluta* and *G. candidum* including agricultural practices, planting of resistant varieties, chemical control, integrated pest management strategies, biological control and plant extracts.

I.4.1 Agricultural practices

Good agricultural practices comprise utilization of plant materials free of pests, crop rotation with non-solanaceous crops, adequate irrigation, removal of plants debris. After

harvest, crop residues should be destroying either by burning or buried as soon as possible (**Retta and Berhe, 2015; Illakwahhi and Srivastava, 2017**). However, *T. absoluta* can retain it population on weeding herbs and regain it host when it is cultivated.

I.4.2 Cultivation of resistant tomato varieties

Developpment of resistant varieties is ongoing (**Guedes and Picanço, 2012; Worku and Sahe, 2018**). Plant resistance is generally associated with the presence of trichome types and densities (**Tissier, 2012**). Trichomes are epidermal structures that originate from the epidermal cells of above ground plant tissues, have been implicated in protection against various biotic attacks, extreme temperature and excessive light (**Mulusew, 2013**). Based on the presence/absence of a glandular, head trichomes can be classified as glandular or non-glandular trichomes. Glandular trichomes are the major sites of different phytochemical production that prevents herbivore attack than non-glandular trichomes. Glandular trichomes are an important source of essential oils that is involved in plant defence to herbivorous (**McDowell *et al.*, 2011**). Then, the resistance could be related with the presence of insect feeding deterrents compounds, present in some wild tomato species that are not present in the common tomatoes species (**Mulusew, 2013**). Recently, research into the possible incorporation of the resistant genes from the wild types into commercial varieties is ongoing (**De Oliveira *et al.*, 2012**). However, the common and most popular tomato varieties currently grown in Cameroon are susceptible to *T. absoluta* infestation and and rot.

I.4.3 Chemical control

From last 50 years, application of chemical synthetic pesticides has been the prevailing control measure for insects and fungi diseases management in crop production. However, the continuous exposure to insecticides including Emamectin benzoate, Acetamiprid, Lambda-cyhalothrine and fungicides like Ridomil, Benomyl and Propiconazole have serious drawback as accumulation of toxic waste on fruits after treatment. Besides, its persistence in the product generates concern in the consumer since fruits are consumed in a relatively short time after harvest. Furthermore, the National Academy of Sciences (NAS) established that synthetic products in foods are carcinogenic and considered to be hazardous to human health. The use and abuse of these compounds can generate resistance in targeted organism and destruction of their natural ennemy. The danger of handling them, due to the possible intoxication and infertility triggered in people who manipulate those compounds. Since they are not readily biodegradable, they tend to persist several years in the environment, considering them as environmental impact products. Moreover, high cost, around 20% of the

production cost is spent on chemicals (**Tzortzakis and Economakis, 2007; Chang *et al*., 2008; Martínez, 2012**).

I.4.4 Traditional Pest Management Practices

The salt is applied on the plants to control stem borer. Moreover, ash is used to protect plants from biting- and sucking-type insects through it repellent effect. In this sense, ash is used in chilli and potato fields to prevent the invasion by aphids. Ash contains silica which interferes with insect feeding and also inhibit fungal development. The spraying of the cow urine on the plants repels insect pests. In addition, planting trap crops or repellent crops on the borders of the field is applied by farmers. Thus, the pest population develops on the trap crops can be killed by using synthetic chemicals or natural control (**Rathore *et al*., 2021**).

I.4.5 Integrated Management strategies

The integrated control method combines many compatible methods to reduce the possibility of attacks by insects and pathogens during the cropping season. These management strategies include cultivation of resistant cultivars, crop rotation, clearing the soil of crop residues, removal of infected plants from the field, application of pheromones traps, application of biological control agents, plant extracts and elimination of the remnants of the crop immediately after harvesting the last fruits (**Afroz *et al*., 2008; Yankova and Ganeva, 2013; Illakwahhi and Srivastava, 2017**).

I.4.6 Biological control

Biological control refers to the utilization of living organisms to reduce the activities and populations of plant insects and pathogens to a non-economic impact level. A multitude of organisms have been implicated as agents responsible for biocontrol of several plants pests and diseases. These so called Biocontrol Agents (BCA) or Bioagents comprise organisms like predators and parasitoids of insects, fungi and bacteria (**Öztemiz, 2013**; **Zappalà *et al*., 2013; Ma *et al*., 2015; Toghueo *et al*., 2016**). However, biological control method required high cost.

I.4.7 Plant extracts

Recent operational approaches to control *T. absoluta* infestation and fungal diseases rot include the development of plant-derived active metabolites with insecticidal and antifungal properties. Then, **De Brito *et al.* (2015)** reported that the ethanolic leaf extract of *Piper amalago* var. *medium* is promising for the control of *T. absoluta* larvae on tomato, since

it exhibits acute toxicity toward these caterpillars and affects the insect's development by reducing its survival and lengthening the larval and pupal stages. Moreover, the works of **Talibi *et al.*** (**2012a**) shown that the methanol extracts of *Cistus villosus, Ceratonia siliqua* and *Halimium umbellatum* exhibited strong antifungal activity against *G. candidum*, the cause of citrus sour rot. Amongst the most credible pesticides sources, essentials oils are presently regarded as a new class of ecological products for controlling insect pest and fungi. Then, the insecticidal and antifungal activities of essential oils are well documented. Indeed, **Adil *et al.* (2015)** reported insecticidal activity of *nigella sativa* essential oils against *T. absoluta* larvae. Moreover, **Tak *et al.* (2015)** shown the contact and fumigant toxicity of *T. vulgaris* and *C. citratus* oil against cabbage and tomato pest, *Trichoplusia ni.* The antifungal activity of *syzygium aromaticum* essential oil against pathogenic fungi of tomato plants have been reported by **Thabet and Khalifa (2018**).

I.5 Essential oils

I.5.1 Definition and location

Essential oils are a complex mixture of plant volatile compounds. Volatiles oils are extracted from plants organs including the flowers, barks, stems, leaves, roots and fruits. The essential oils are biosynthesized and accumulated in specialized cells like glandular trichome and osmophores. They are extrracted by various methods (**Ali *et al.*, 2015**).

I.5.2 Extraction methods

Before extraction, the plant material must be subjected to postharvest treatment such as chopping, crushing, drying which make essential oils more extractable and increases the yield of extraction (**Fokou *et al.*, 2020**). Essential oils are obtained from plant raw material by several extraction methods including hydrodistillation and expression (**Fokou *et al.*, 2020**). Therefore, the most commonly used is hydrodistillation (using at 80%) as this method is efficient and easy to implement (**Ndoye, 2001**). Essential oils are stored in a cool, dry space and in air-tight amber glass containers.

I.5.3 Chemical composition and biosynthesis

The volatile organic compounds which constitute EOs originate from three categories of chemicals: phenylpropanoid derivatives, fatty acid derivatives, and isoprenoids (Figure 5)

(**Rehman *et al.*, 2016**). Terpenoid and phenylpropanoid derivatives are the main components found in essential oils (**Fokou *et al.*, 2020**).

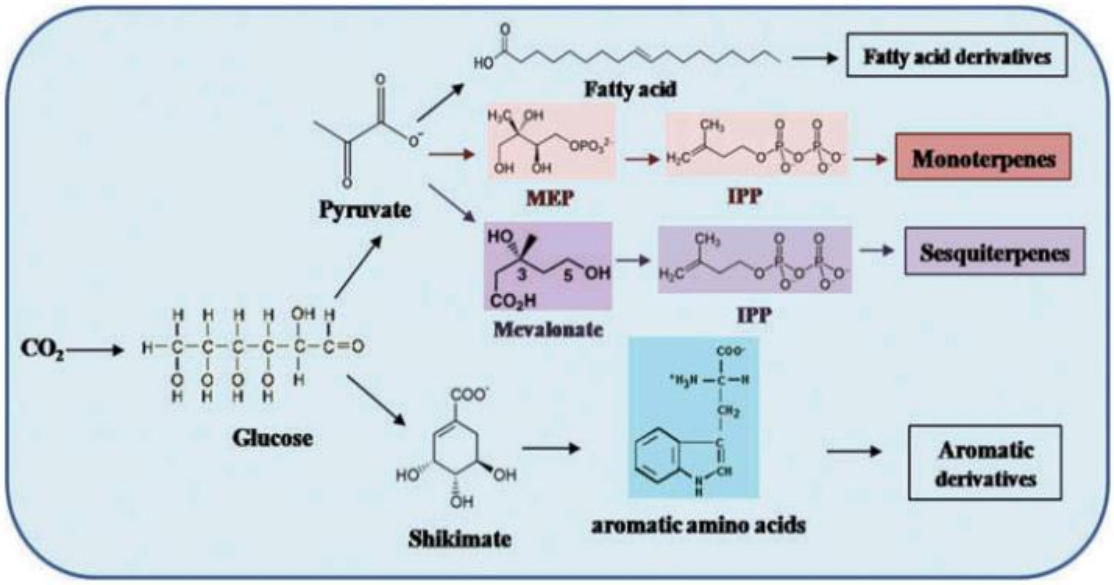

Figure 5: Biosynthesis pathways of volatile compounds of essential oils

MEP : 2-methyl-erythritol-4-phosphate; IPP : isopentyl pyrophosphate.

I.5.4 Biological activity of essential oils

Volatile oils help to protect the plants by their antibacterial, antiviral, insecticidal and antifungal properties. Numerous studies have demonstrated the efficiency of essential oils against insects and fungi which hinder crops. In fact, the bioactivity of five Citrus essential oils against the stored product pest, *Tribolium confusum* was reported by **Campolo *et al.*, (2014)**. In addition, **Alam *et al.*, (2017**) shown the efficiency of *Thymus capitatus* EO against *T. absoluta* and fungi associated to tomato rot as *Fusarium oxysporum*, *Aspergillus niger*, *Alternaria alternata*, *Botrytis cinerea* and Penicillium sp. The activity of EOs of *Zanthoxylum xanthoxyloides* and *Syzygium aromaticum* was highlighted against *Phytophthora megakarya* associated to cocoa black pod disease (**Nana *et al.*, 2015**).

I.5.5 Mechanism of action of essential oils on insect

To cope with bioactive secondary metabolites, the insects can decrease the sensitivity of the target site of pesticides in the nervous system such as γ-aminobutyric acid (GABA) receptors, octopamine receptors and the nerve conduction enzyme acetylcholinesterase (AChE) (**Bezerra da Silva *et al.*, 2016**; **Jankowska *et al.*, 2018**; **Senthil-Nathan, 2020**). Otherwise, the insects can use a variety of detoxifying enzymes including glutathione S-transferase, superoxide dismutase and esterases (**Li *et al.*, 2013**). Thus, the study of the

neurotoxic action and the determination of the activity of detoxifying enzymes are currently the main aspects to study the mechanism of action of essential oils (**Potter and Wadkins, 2006**; **Mohamed *et al.*, 2016**). EOs are estimated to be a potential source of insecticides due to their ability to modifying the insect AChE activity (**Jankowska *et al.*, 2018**). AChE is known as a target enzyme for insect control. Preliminary works suggested that this enzyme may be the main target of the essential oil, and the decline of the AChE enzyme activity could be one of the main reasons that caused the death of the insects (**Liao *et al.*, 2017**). AChE is one of the most important enzymes in neuro-neuronal and neuromuscular junctions in both insects and mammals (**Jankowska *et al.*, 2018**) which plays a crucial role in the maintenance of normal transmission of neural impulses in synaptic clefts, by catalysing the hydrolysis of the neurotransmitter acetylcholine into acetate and choline (**Bezerra da Silva *et al.*, 2016**; **Kim *et al.*, 2015**). The anti-acetylcholinesterase activity of EOs creates the build up of concentration of the acetylcholine in the synapse, so that the post-synaptic membrane is in a state of permanent stimulation which results in hyperexcitability and paralysis leading to death (**Begum *et al.*, 2011**; **Lionetto *et al.*, 2013**; **Rajashekar *et al.*, 2014**).

I.6 Generalities on interested plants

I.6.1 Generalities on Lamiaceae family

Lamiaceae family is particularly important to humans for it flavour, fragrance, or medicinal properties. It is distributed nearly worldwide with 236 genera and more than 7.000 species as *Thymus vulgaris*.

I.6.1.1 *Thymus vulgaris*

I.6.1.1.1 Ecology and botanical description

Thymus vulgaris Linnaei (1753) is a tiny perennial shrub, flowering plant in the mint family Lamiaceae (**Prasanth *et al.*, 2014**). *T. vulgaris* grows well during a temperate to heat, dry, sunny climate. It prefers well drained soils with a pH of 5.0 to 8.0. It is growing up to 15-30 cm tall by 40 cm wide (**Kindersley, 2008**). The stems become woody with age. *T. vulgaris* leaves are oval to rectangular in form, little, usually 2.5 to 5 mm long and vary signifcantly in form and hair covering, depending on the variety (**Prasanth *et al.*, 2014**).

Taxonomic Classifcation of *Thymus vulgaris* (ITIS, 2021a)

Kingdom: Plantae

Division : Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus : Thymus L.

Specie : *T. vulgaris* L.

I.6.1.1.2 Traditionnal uses and previous works

Thymus vulgaris is used as stimulant, tonic and to treat fever, diarrhoea, infected wounds and cough (**Khafaji, 2018**). Previous work reported antidiabetic, antitumoral, antiviral, antibacterial, antioxydant, antiinflamatory, insecticidal and antifungal activities of *thymus vulgaris* oil (**Prasanth *et al.*, 2014; Khafaji, 2018**). In fact, **Tak *et al.*, (2015)** shown the contact and fumigant toxicity of *T. vulgaris* oil against third instar larvae of cabbage and tomato pest, *Trichoplusia ni*. The LD_{50} was 54 µg/larvae and 200.8 µg/mL air by contact and fumigation assay respectively. Moreover, **Nguefack *et al.*, (2009)** reporded the antifungal activity of *thymus vulgaris* oil against *Aspergillus ochraceus*, *Penicillium expansum* and *Penicillium verrucosum*. The authors reported that the antifungal activity determined and expressed as a Number of Decimal Reduction of the colony forming units per mL (NDR cfu/mL) shown highest activity at pH 3 against *Aspergillus ochraceus* (0.63 NDR cfu/mL) and at pH 9 against *Penicillium expansum* (0.40 NDR cfu/mL) and *Penicillium verrucosum* (> 6 NDR cfu/mL). The antifungal effect of *T. vulgaris* oil on *Geotrichum citri-aurantii* arthroconidia germination and germ tube elongation was expressed by **Liu *et al.*, (2009**). At 600 µL/mL, *T. vulgaris* oil inhibited the germination of about 94% of the arthroconidia and the germ tube length was only 4.32 µm.

I.6.2 Generalities on Poaceae family

Poaceae, alternative name Gramineae are one of the most ecologically and economically important plant families (**Stanley, 1999**). This family contains 800 genera and 11000 species worldwide including *Cymbopogon citratus* (**Khan *et al.*, 2019**).

I.6.2.1 *Cymbopogon citratus*

I.6.2.1.1 Ecology and botanical description

Cymbopogon citratus (DC.) Stapf (1906) is widely grows in tropical and subtropical lands of the Indian subcontinent, South America, North America, Africa, Australia and Europe (**Skaria *et al.*, 2006**). The grounds on which it is cultivated are mostly infertile and wastelands. The plant generally grows up to 1.8 m high and 1.2 m in width. It has a small rhizome, and the leaves appear from the soil directly without any stem. The leaves are 1.3–2.5 cm in width and around 1 m in length (**Shah *et al.*, 2011**).

Taxonomic Classifcation of *Cymbopogon citratus* (ITIS, 2021b)

Kingdom: Plantae

Division : Tracheophyta

Class: Magnoliopsida

Order: Poales

Family: Poaceae

Genus : Cymbopogon

Specie : *C. citratus*

Synonyms: *Andropogon citratus* (DC.) ex Nees (1813); *Cymbopogon nardus* (L.) Rendle, (1899)

I.6.2.1.2 Traditionnal uses and previous works

Initially, *C. citratus* was used to flavour foods in Thailand and Vietnamese cooking. It has a beneficial use in African and South American regions for flavouring tea (**Haque *et al.*, 2018**). It is also popular in alcoholic and non-alcoholic drinks (**Abdulazeez *et al.*, 2015**). The pleasant lemon fragrance of this grass has long been used in perfumery and related cosmetics, as well as food industries (**Ranade and Thiagarajan, 2015**). There are numerous examples of the application of *C. citratus* for health remedies by different ethnic groups (**Ravinder *et al.*, 2010**). For instance, tea made from *C. citratus* leaves is predominantly used as an antispasmodic, analgesic, diuretic and anti-inflammatory compound (**Leite *et al.*, 1986; Formigoni *et al.*, 1986**). It is used for lowering blood pressure and treating catarrhe and rheumatism, as well as used to cure the sore throat (**Haque *et al.*, 2018**). In addition, a number

of biological properties of *C. citratus* are reported over the years, including but not limited to antibacterial, antiprotozoal, anti-inflammatory, antioxidant, anti-carcinogenic, cardio-protective, anti-rheumatic, insecticidal and antifungal activities (**Ekpenyong *et al.*, 2015; Rani *et al.*, 2019**). Indeed, **Tak *et al.*, (2015)** shown the contact toxicity of *C. citratus* oil on third instar larvae of *Trichoplusia ni* with LD_{50} value of 123.8 µg/larvae. In addition, **Yousef, (2013)** reported highly antifungal activity of *C. citratus* oil, as it showed the lowest MIC value of 5 µL/0.4l air space on mycelia growth of *Aspergillus niger* and *Aspergillus fumigatus* and 10 µL/0.4l air space on spore germination of both fungi. In addition, **Tzortzakis and Economakis, (2007)** have shown that spore production depressed by 70% for *Botrytis cinerea*, 58% for *Colletotrichum coccodes*, 41% for *Aspergillus niger*, 40% for *Cladosporium herbarum*, and 35% for *Rhizopus stolonifer* at 25 ppm due to *C. citratus* oil activity.

MATERIAL AND METHODS

CHAPTER II: MATERIAL AND MEHODS

II.1 Survey on the occurrence of *Tuta absoluta* in Foumbot production basin

II.1.1 Study site information

This study was conducted in Foumbot through the Noun division, in the western highlands located in the agroecological zone III of Cameroon, the major area of tomato production (Figure 6) (**MINADER, 2009**; **MINADER, 2015**).

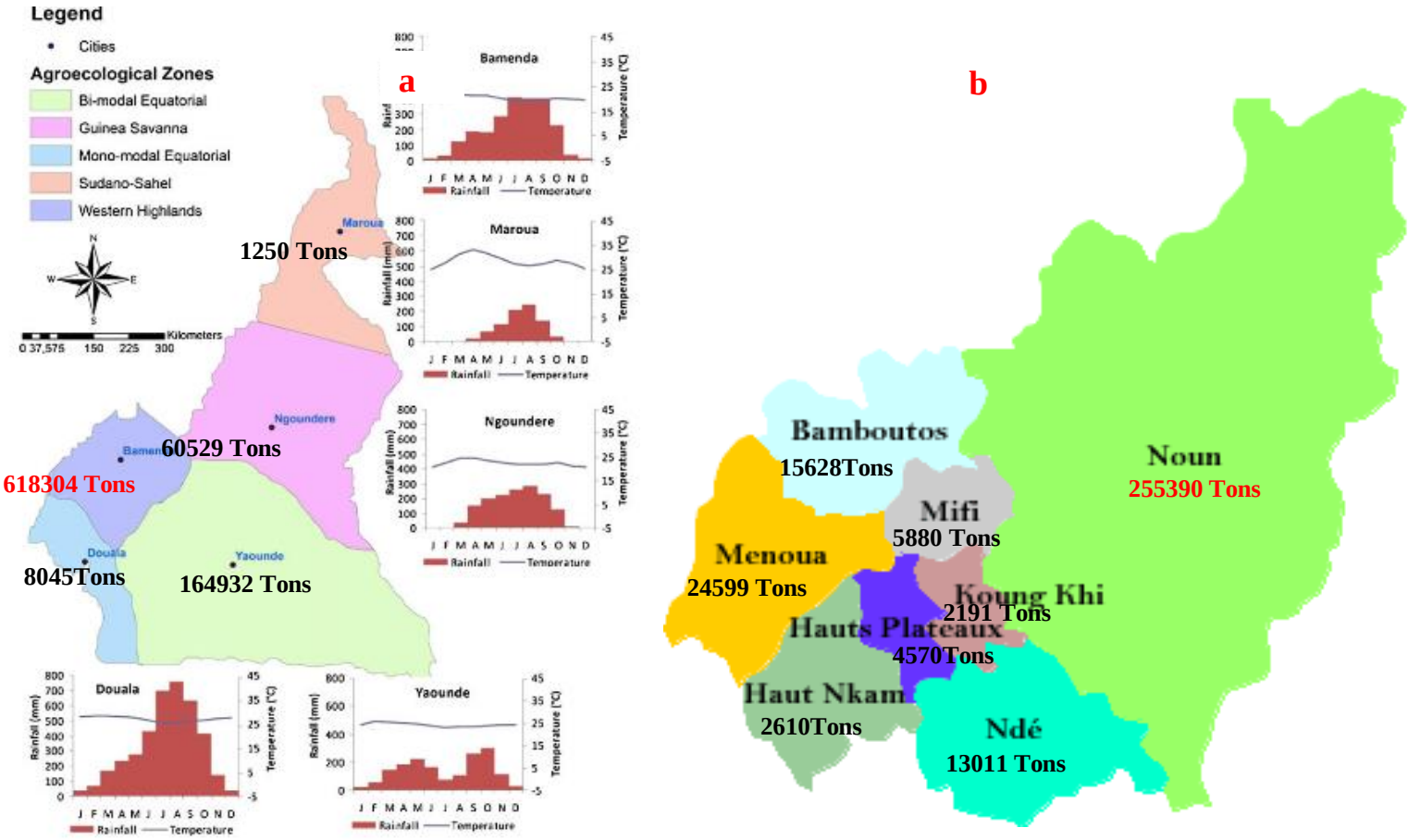

Figure 6: Agroecological zones of Cameroon (a) and divisions of the Western highlands (b) where tomato is produced

Foumbot is located between longitude 10°37'57.00" E and latitude 5°30'28.91" N in the western highlands. The means annual temperature and relative humidity are 21°C and 80% respectively. The main soil type is volcanic with high agronomic value. The climate is charactarized by a long rain season from mid-March to mid-November followed by a short

dry season from mid-November to mid-March. Annual rainfall ranged from 2500 to 5000 mm with minima and maxima in November and July respectively (**Commune de Foumbot, 2014**). Tomato is grown in rainy season as well as in dry season. The survey was conducted in dry season as it is favourable for the optimal development (30°C) of *T. absoluta*, associated with highest incidence and severity. Survey was applied in five villages with mainly commercial production: Mangoum, Fossang, Fosset, Mougnie and Mbantou (Figure 7).

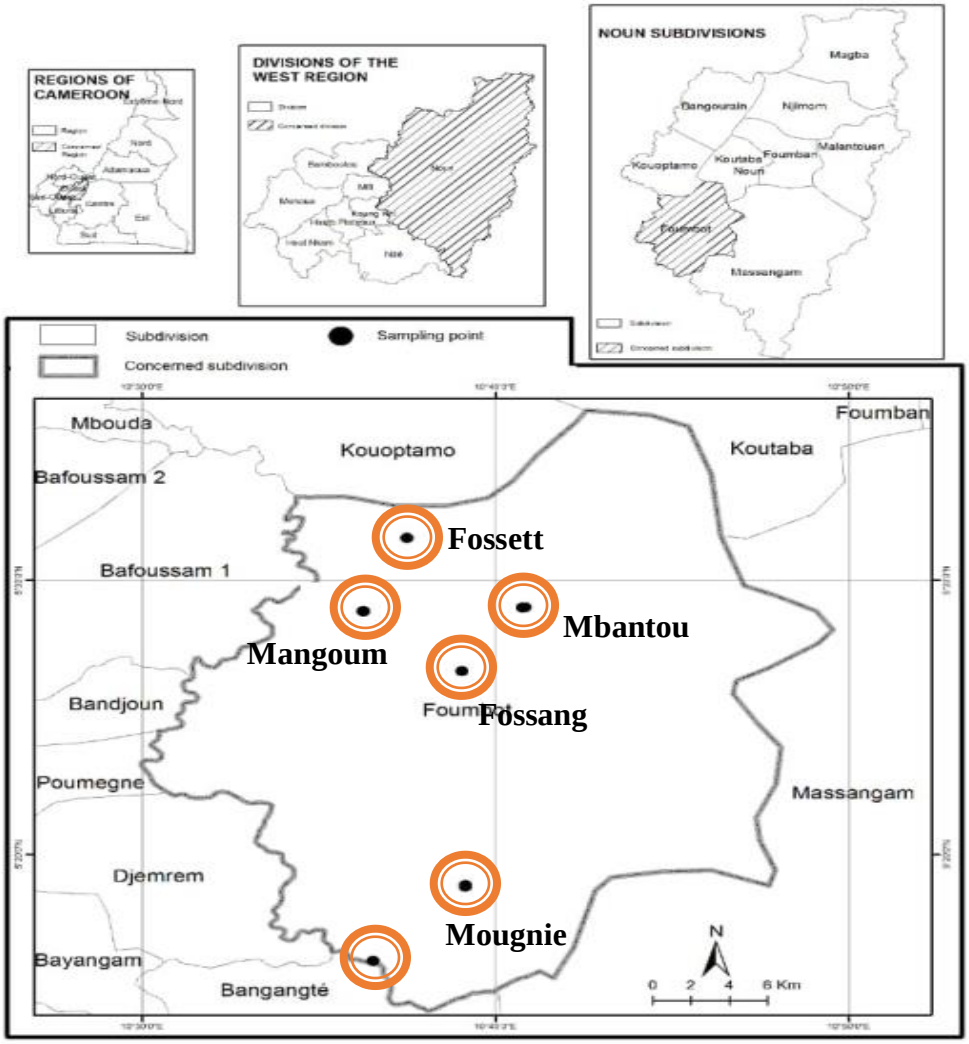

Figure 7: Survey site and sampling points in Foumbot production basin

II.1.2 Data collection procedure

Data on the occurrence of *T. absoluta* was collected following a questionnaire (Appendice 1) and field sampling.

II.1.2.1 Data collection following questionnaire

Random sampling of fifty-six (56) tomato growers both males and females were interviewed individualy or in groups (Figure 8) following open and close questions. The objective of the questionnaire was to assess farmer's perception of *T. absoluta* in Foumbot production basin (Appendice 1). The informations were collected on the characteristics of growers, the knowledges of farmers about *T. absoluta* and fields characteristics. The questionnaire was firstly developed and pretested.

Figure 8: Individual (a) and group (b) interviews with tomato farmer's in the Mbantou village

II.1.2.2 Data collection by fields sampling

Nineteen commercial farms (Table 2) of differents growth stage (vegetative period, flowering, early fruiting and fruit maturity) and tomato varieties (local and hybrid genotypes) were randomly selected across the Foumbot subdivision at intervals of 2 to 3 km apart. Fields were assessed to determine incidence and severity of *T. absoluta* infestation. The disease estimation was conducted following the "W sampling design" (**Delp *et al.*, 1986**): the design (W) was divided into four (4) uniform sections (Figure 9). Then in each section, a quadrat of 1 m^2 was drawn randomly. Within each quadrat, the number of tomato plants showing symptoms associated to *T. absoluta* attacks was taken as percentage over the total number of plants. Damages were characterized by larvae mediated-mines under leaves cuticles, feeding of plants aerial parts with frass (fine powdery material that plant-eating insects pass as waste after they digest plant parts) production, folding, chlorosis and necrosis of leaves, drying and flowers abortion, reduce size, puncture marks, holes promoting rot due to secondary infective

agents on fruit, wilt and death of plants (**Ballal *et al.*, 2016**; **Rwomushana *et al.*, 2019**). The mean of incidence from each quadrat was the incidence in the farm (**Mwang'ombe *et al.*, 2007**). Data on the severity of plant damage was rated using the 1–5 rating scale of **Ayalew (2011**) based on the percentage of plant parts showing described symptoms (Table 3). After field sampling, tomato leaves infested by *T. absoluta* larvae were collected and stored in plastic containers for production of experimental material in the laboratory.

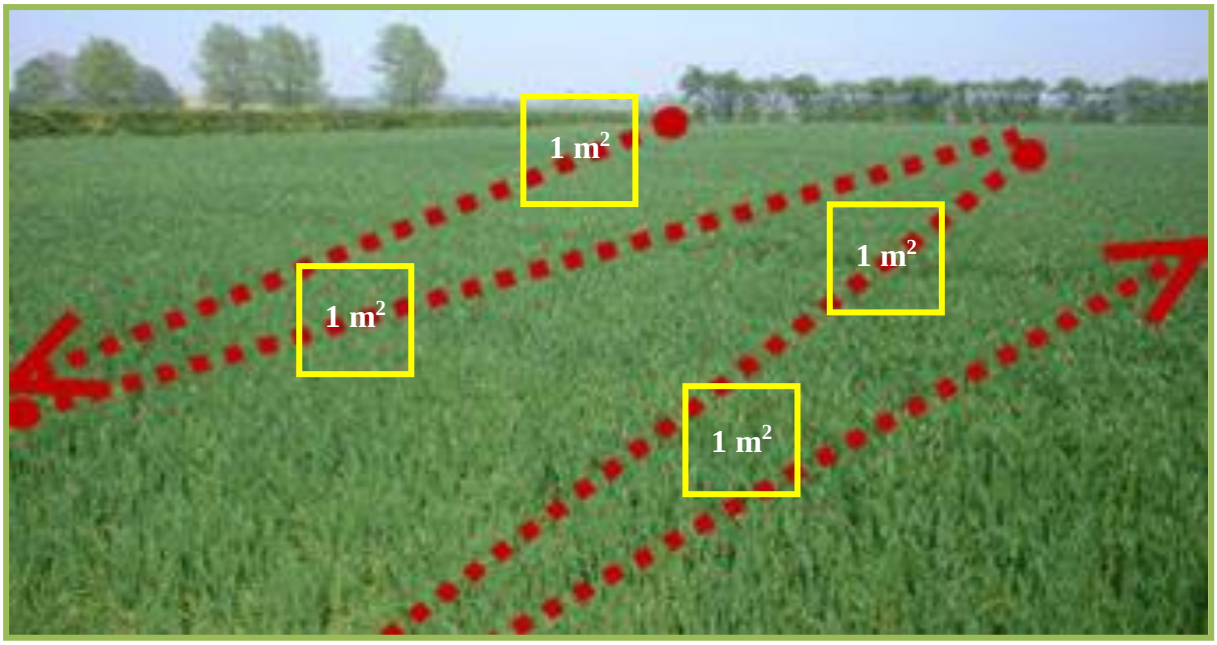

Figure 9: W sampling design

Table 2: Global Positioning System localisation of the sampled commercial farms in Foumbot production basin

Farms	Latitudes	Longitudes	Altitudes
Farm 1	5°15'51.667" N	10°36'26.638"E	971.5m
Farm 2	5°18'52.317" N	10°39'09.409"E	943.5m
Farm 3	5°18'51.703" N	10°39'08.587"E	911.5m
Farm 4	5°18'48.731" N	10°39'08.337"E	944.5m
Farm 5	5°18'49.345" N	10°39'09.159"E	912.5m
Farm 6	5°26'41.045" N	10°39'02.996"E	977.5m
Farm 7	5°31'33.188" N	10°37'29.321"E	1069.5m
Farm 8	5°29'23.814" N	10°34'49.523"E	1049.5m
Farm 9	5°29'22.165" N	10°34'48.834"E	1044.5 m
Farm 10	5°29'18.219" N	10°34'46.144"E	1019.5m
Farm 11	5°29'24.140" N	10°35'01.675"E	1014.5 m
Farm 12	5°29'13.652" N	10°35'02.217"E	1025.5m
Farm 13	5°29'23.652" N	10°35'01.317"E	1020.5m
Farm 14	5°29'33.347" N	10°35'01.975"E	1025.5m
Farm 15	5°29'38.570" N	10°35'06.849"E	1012.5m
Farm 16	5°29'41.601" N	10°35'00.495"E	1034.5m
Farm 17	5°29'28.512" N	10°35'06.557"E	1002.5m
Farm 18	5°29'01.443" N	10°40'49.703"E	995.5m
Farm 19	5°29'00.447" N	10°40'45.959"E	1000.5m

Table 3: Rating scale of evaluation of *Tuta absoluta* severity

Rating scale	Symptoms
1	0-5% plant organs infested
2	6-20% plant organs infested
3	21–40% plant organs infested
4	41–60% plant organs infested
5	> 60% plant organs infested

II.2 Assessment of the insecticidal potential of *Thymus vulgaris* and *Cymbopogon citratus* essential oils against *Tuta absoluta*

II.2.1 Plants collection and essential oils extraction

II.2.1.1 Plants collection

Fresh aerial organs of *T. vulgaris* (2.2 kgs) and *C. citratus* (1.5 kgs) (Figure 10) were collected in November 2017 at 8 am in Mbouda (5°40′11.99″ N, 10°3′2.16″ E) and Oyom-abang (3°52′22.22″ N, 11°28′48.86″ E) respectively. The taxonomy of plants was confirmed at the National Herbarium of Cameroon where voucher specimens were deposited under the reference numbers: 42851/CAM for *T. vulgaris* and 48536/SRF/CAM for *C. citratus*.

Figure 10: Aerial parts of *Thymus vugaris* (a) and *Cymbopogon citratus* (b)

II.2.1.2 Essential oils extraction

Plant samples were subjected to hydrodistillation using a Clevenger-type apparatus (**Clevenger, 1928**) for approximately 6h (Appendice 2). The resulting essential oils were

collected by decantation, dried through anhydrous sodium sulfate and stored in dark glass vials until use. The yield of extraction was calculated relative to the mass of the starting plant material as follows:

Yield (%w/w) = [(Mass of essential oil (g)/ Mass of vegetal material (g)) x 100].

II.2.2 Evaluation of the chemical composition of essential oils

The chemical composition of the essential oils was analysed by Gas Chromatography (GC) coupled with Mass Spectrometry (MS) using a GC-MS-HP 6890 equipment (Agilent Technologies, 5977A MSD) and 7890B GC System Chemetrix (pty) Ltd, DE (Germany). The GC-MS apparatus was equipped with a Zebron-5MS column with 5% phenylmethylpolysiloxane as stationary phase. One µL of each essential oil prepared at 0.25 µL/µL in n-hexane was injected into the apparatus, using the Split mode at 5:1 ratio. Helium was used as carrier gas at a flow rate of 2 mL/min. The ionization voltage was set at 70 eV. The injector temperature was set at a static value of 280°C while the oven temperature was set to vary from 70°C to 270°C with the following consecutive gradients: (1) from 70°C to 120°C with an increment of 15°C/min; (2) from 120°C to 180°C with an increment of 10°C/min and finally (3) from 180°C to 270°C at 20°C/min. The components of the essential oils were detected through a mass selective detector (HP5973). The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements. The identification of the constituents was assigned on the basis of comparison of their retention indices and their mass spectra with those given in the literature (**McLafferty and Stauffer 1989**; **Adams 1995**; **Joulain and König 1998**) and the Nist11 library database with Xcalibur software.

II.2.3 *Tuta absoluta* and tomato plants rearing

Tuta absoluta was reared using the protocol described by **Ferrari de Brito *et al.*,** (**2015**), with little modifcations. Briefy, the tomato (cv. Rio Grande) plants were grown from seeds at the experimental field of the Antimicrobial and Biocontrol Agents Unit (AmBcAU), University of Yaoundé I, Cameroon and used to rear *T. absoluta* for subsequent assessment of knockdown and insecticidal activities of essential oils. Tomato leaves bearing *T. absoluta* larva were collected in an humidified plastic box in commercial tomato farm in Foumbot (West Region of Cameroon; 5º29’00.447” N, 10º40’45.959”E). Larva were thereafter transferred individually into Petri dishes with fresh tomato leaves from the AmBcAU

experimental pesticide-free garden, with daily re-supply until adult emergence. Adults were then released into an egg-laying cage (80×160×60 cm) housed with potted tomato plants for oviposition (Figure 11). The laboratory conditions were: 25 ± 2°C, 65 ± 5% RH, and photoperiod of 16/8 hours light to dark cycles. The adults were provided with food source by soaking cotton wool in 10% honey solution and placing it in rearing cages. After egg hatching, the frst instar larva were collected individually into Petri dish on fresh tomato leaves and maintained until the fourth larval stage which was used for the bioassays.

Figure 11: Moths releasing cage for egg-laying (a) on tomato plants (b)

II.2.4 Preparation of stock solutions of essential oils and reference insecticides

The tested concentrations of *T. vulgaris* and *C. citratus* essential oils were prepared using Tween 80 (Sigma Aldrich, Munich, Germany). Essentially, two-fold dilutions were performed in order to obtain test oil concentrations of 0.4 µL/mL; 0.8 µL/mL; 1.6 µL/mL; 3.2 µL/mL and 6.4 µL/mL and each tested for direct contact and fumigant toxicity activities. For individual assays, 10 µL of each oil concentration were dispensed per larvae or per disc. The concentration range used was chosen based on preliminary tests. The reference insecticide Lynx® (λ-cyhalothrin 15g/L + acetamiprid 20g/L) (**MINADER, 2019**) was prepared according to the manufacturer's instructions (Sun valley Hall Limited, Hong kong) at a single dose by adding 3.33 mL of the conditioned liquid insecticide form to 996.67 mL of sterile distilled water so as to get a final concentration of 3.33mL/L which is the recommended dose for farm application against *T. absoluta*.

II.2.5 Assessment of the insecticidal effects of the essential oils on *Tuta absoluta* larvae

II.2.5.1 Contact toxicity assay

The essential oils were evaluated through contact toxicity against *T. absoluta* larva following the procedure described by **Slimane *et al.*, (2014)**. For each contact toxicity assay, 10 larva at the fourth stage were placed on fresh tomato leaves into a Petri dish (9 cm Ø), then, 10 µL of each test concentration of essential oil were drop-inoculated onto the larvae using a [2-20 µL Eppendorf] micropipette (Sigma Aldrich, Munich, Germany). Each experiment was performed in quadruplate and the concentrations tested were 0.4 µL/mL; 0.8 µL/mL; 1.6 µL/mL; 3.2 µL/mL and 6.4 µL/mL. Petri dishes were immediately sealed with parafilm and maintained in rearing conditions as described above. The control plates were provided with 10 µL of Tween 80 as negative control and 10 µL of Lynx® as positive control. Each experiment in quadruplate was repeated twice on different days. The plates were subsequently examined after four hours and the number of knocked down larva (immobilized) (**Sawicki, 1962**) was recorded and expressed in terms of percentage over the initial number of larva per treatment as follows:

Knockdown (%w/w) = [(Number of knocked down larva / total number of larva) x 100]

Also, the doses required to knock down 50% and 90% (KD_{50} and KD_{90}) of larva were determined. Thereafter, the knocked down larva were transferred onto fresh tomato leaves and incubated for 24 more hours under the same rearing conditions, but without treatment. The larva which could not react to the pressure of the brush upon the incubation period on fresh tomato leaves were considered as dead (**Samira and Habib, 2017**). The percent mortality rate was then calculated and the doses required to kill 50% and 90% (LD_{50} and LD_{90}) of larva were also determined.

The surviving larva were inspected daily to assess the effect of essential oils on stage transition (larvae-pupae-adult). Thus, they were transferred individually into Petri dishes containing tomato leaves and incubated till adult emergence. Then, larval and pupal duration periods were recorded.

II.2.5.2 Fumigant toxicity assay of the essential oils

Fumigant toxicity of essential oils was assessed following the method described by **Samira and Habib (2017)**. Essentially, 10 larva at the fourth stage per Petri dish (9 cm Ø)

were placed on fresh tomato leaves. Whatman No. 1 filter paper disk was fixed onto the inner surface of each plate lid and impregnated with 10 µL of each test concentration of essential oil using the micropipette. The tested concentrations were 0.4 µL/mL; 0.8 µL/mL; 1.6 µL/mL; 3.2 µL/mL and 6.4 µL/mL. Quadruplate Petri dishes for each concentration were immediately sealed with parafilm and maintained in rearing conditions as described above. The control plates were provided with 10 µL of Tween 80 as negative control and 10 µL of Lynx® as positive control. Each experiment was repeated twice on different days. The activity data were recorded as described in contact toxicity assay above. The knockdown doses (KD_{50} and KD_{90}) and lethal doses (LD_{50} and LD_{90}) as well as the mortality rate were determined as above. Surviving larva were further observed daily under the same rearing conditions up to larval transition to pupae and to adult.

II.2.6 Determination of the mecanism of action of essential oils: anti-acetylcholinesterase activity

II.2.6.1 Extraction of Acetylcholinesterase from *Tuta absoluta* larvae

Acetylcholinesterase extraction was performed as described by **Podolska and Nadolna, (2014**). Then, larva (10% w/v) were homogenized in plastic tube using a mechanical blender in ice-cold phosphate buffer (0.05 M, pH7.4). Homogenate was centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was collected with pipette and stored at -80 °C until inhibition assay. Protein concentration was determined as described by **Bradford (1976)**, using Bovin Serum Albumin (BSA) as the standard.

II.2.6.2 Determination of protein content

Twenty-five microliters of phosphate buffer (0.05 M, pH7.4) were added to twenty microliters of distilled water. Then, five microliters of enzyme extract and one hundred microliters of protein reagent (solution of Coomassie Brilliant Blue G-250) were added. The mixture was mixed by vortexing and the absorbance at 595 nm was measured after 10 min against a blank prepared with phosphate buffer instead of enzyme extract. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve from pure BSA at concentrations range of 0 to 100µg/mL. the protein content was expressed as microgram equivalent BSA per gram fresh weight of larvae.

II.2.6.3 Anti-acetylcholinesterase activity

The anti-acetylcholinesterase assay of EOs was performed using the procedure of **Ellman et *al*., (1961)**. Therefore, 10 µL of enzyme solution were transferred to each of the 96-wells plate further mixed with 20 µL of EO prepared in tween 80 to achieve final concentrations of 0.4 µL/mL; 0.8 µL/mL; 1.6 µL/mL; 3.2 µL/mL and 6.4 µL/mL. Then, 150 µL of cold phosphate buffer (4°C; 0.05 M; pH7.4) were added. The positive control was prepared with Lynx as acetylcholinesterase inhibitor and the negative control with tween 80. The plates were incubated at 25°C for 10 min. This was followed by addition of 20 µL of Dithiobisnitrobenzoate (DTNB) at the final concentration of 0.3 mM and 20 µL of substrate acetylthiocholine iodide (ACTHI) at 0.4 mM. Yellowish or colourless solution was observed during reaction for 30 minutes at room temperature then; absorbance was recorded on a microplate reader at 412 nm. The inhibition percentage (%) was determined as follow*:*

% inhibition = [(Absorbance of the control - Absorbance of the test sample) / (Absorbance of the control) x 100].

The IC_{50} was determined using GraphPad Prism 5.0. The specific activity of acetylcholinesterase was expressed as ΔOD (412 nm)/min/µg protein.

II.3 Antifungal activity of *Thymus vulgaris* and *Cymbopogon citratus* essential oils against *Geotrichum candidum* associated to *Tuta absoluta* infestation

II.3.1 Fungal isolation

Tomato fruits infested by *T. absoluta* and presenting typical sour rot characteristic like soft tissues smelling sour odour, whitish mycelia and liquid secreting on crack point were collected from tomato farms in plastic boxes lined with wet Buvard paper and transported to the laboratory. Fungal isolation was performed using the protocol described by **Lemma *et al.*, (2014)**. Thus, small pieces (~ 3 mm diameters) were cut at rotting edges with a forceps and deepped in 1% sodium hypochloride for 2 min and rinsed three folds with sterile distilled water. The air-drying fragments were inoculated on PDA medium supplemented with chloramphenicol (500 mg/L) and incubated at 25±2°C during 3 days. Emerging fungi were subcultured into fresh PDA medium under the same above conditions until pure colonies are obtained for the pathogenicity test.

II.3.2 Pathogenicity test

II.3.2.1 Preparation of pathogens suspension

From 5-day-old culture plates of isolates incubated at 25±2°C, conidia were harvested by flooding PDA plates with 5 mL of physiologic liquid (0.9% of NaCl). The obtained solution was centrifugated at 2000 round per minute (rpm) for 2 minutes. Then, the supernatant was collected and the charge of the conidia was determined using an haemocytometer and adjusted to 10^6 conidia/mL with physiologic solution.

II.3.2.2 Artificial inoculation of wounded fruits

The pathogenicity of the isolated fungi was assessed as described by **Moline, (1984)**. Briefly, healthy fresh tomato fruits (cv. Rio Grande), were washed under tap water, sterilized by deepping in 1% sodium hypochloride for 2 min and rinsed three folds with sterile distilled water. The air-drying fruits were punctured with sterile needle to a depth of 3 mm at the equatorial side and placed in a plastic box lined with wet, sterilized Buvard paper. Thus, 20µL of conidia suspension at 10^6 conidia/mL were inoculated on wounded surface which were covered with humidified cotton wool. The physiologic solution served as negative control. Another control was prepared without conidia suspension and physiologic solution. Fruits were stored at 25±2°C for 5 days. Three replications were prepared and the experiment was repeated twice. Fruits were observed daily for rot development. After incubation, disease incidence was determined as the percent ratio between the numbers of rotten wounds per total number of wounds (**Talibi *et al.*, 2012b**). The transverse section of each fruit was cut along the plane of inoculation and the diameters of fruit rot for each fungal isolated were meaured from inoculation points (**Oladiran and Iwu, 1993**). The virulence status was determined following the scale from **Chehri, (2015)** as follow:

a = no visible symptom (nonvirulent);

b = 5–10 mm^2 of rotted area (hypovirulent);

c = 11–20 mm^2 of rotted area (moderately virulent); and

d= rotted area ≥ 21 mm^2 (high virulent).

The re-isolations were made by culture diseased tissues onto PDA and the resulting colonies identified to complete Koch's postulate.

II.3.3 Identification of pathogens

II.3.3.1 Morphological identification

The morphology was observed by using special keys of identification (**De Hoog *et al*., 1986).** Macroscopic observation was based on coloration of colony on right and reverse side of Petri dish, aspect and colony diameter after 10 days of growth. While microscopic observation was focused on the coloration, aspect and wide of hypha; conidiogenesis; aspect and shape of conidia.

II.3.3.2 Molecular identification

The sequencing of the ITS1-5.8S rDNA-ITS4 region was used to confirm identity from Morphological tools. Molecular identification was performed through DNA Extraction, DNA amplification and sequencing.

II.3.3.2.1 Genomic DNA extraction

Desoxyribonuclic acid was extracted from mycelial fragments scraped from the surface of culture plates of 7 days old at 25±2°C using a commercial kit (NucleoSpin^{R} Plant II) as recommended by the manufacturer (MACHEREY-NAGEL, www.mn-net.com).

II.3.3.2.2 Desoxyribonuclic acid amplification of ITS

DNA was amplified by Polymerase Chain Reaction (PCR) with universal primers ITS1 and ITS4. The internal transcribed spacer (ITS) of nuclear ribosomal DNA was used. The ITS1-5.8S rDNA-ITS4 region was amplified using PCR in a system including 10 µl of Dream Taq Green Master Mix (2%), 2 µl of primers ITS1(5µm), 2 µl of primers ITS4 (5µm), desoxyribonucleotide triphosphate, 4.6 µl deionized distilled water and 2 µl of DNA templates. PCR conditions were 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds (denaturation), 50°C for 45 seconds (annealing) and 72°C for 1 minute after which the reaction was kept at 72°C for 10 minutes (extension).

II.3.3.2.3 Agarose gel electrophoresis

To check if the PCR was carried out effectively, the gel electrophoresis was performed using 1% agarose gel. Indeed, one hundred milliliters (mL) agarose gel was prepared by dissolving 1g of agarose in 100 mL of Tris-acetate Ethylenediaminetetraacetate (TAE) at 1% and the mixture was dissolved by heating. Ten µL of SYBR SAFE (10000%) was added to allow visualization of DNA bands. The voltage was set at 70 volts for 5 minutes at 40 mA

then, 100 volts for 30 minutes at 40 mA. The gel was exposed to ultraviolet light under the transluminator and the DNA fragments were visualized.

II.3.3.2.4 Purification and sequencing

PCR amplicons were purified by mixing 10 µL of samples with 4 µL of clean sweep PCR purification enzyme (Applied Biosystems by Thermo Fisher Scientific, Lithuania). Purification conditions were 37°C for 15 minutes then 18°C for 15 minutes.

The BLAST (Basic Local Alignment Search Tool) algorithm were used to screen the NCBI (National center for Biotechnology Information) /Genbank database of fungal nucleotide sequences to identify ITS sequences homologous to those obtained from test fungi isolate. A sequence similarity dendrogram was developed with the nucleotide sequences of the isolates with those of references strains available in NCBI genbank.

II.3.4. Antifungal assay of *T. vulgaris* and *C. citratus* essential oils against *G. candidum*

II.3.4.1 Mycelia growth inhibition assay

The inhibition of mycelia growth was performed using the food poisoning method (**Lahlou, 2004**). In prelude, a precised volume of stock essential oils solutions prepared in Tween 80 (1:9 V/V) was added aseptically to a fixed volume of sterile Potato Dextrose Agar (PDA) medium (~45°C) prepared with chloramphenicol according to the manufacturer's inctructions (Appendice 3). The mixture was done to achieve concentrations varying from 0.09 to 1.42 µL/mL and from 0.35 to 5.67 µL/mL respectively for *C. citratus* oil and *T. vulgaris* oil based on the preliminary test. The resulting growth media were poured (20 mL/plate) into sterilized Petri dishes (9 cm diameter). After its solidification, Petri dishes were inoculated with a mycelia disc of 8 mm diameter taken from the growing edges of 4 day old cultures. In controls, Propiconazole Tilt 25 EC was used as positive control at concentrations varying from 0.02 to 0.35 µL/mL. Tween 80 served as negative control and PDA medium was used as blank. All Petri dishes were incubated from 8 to 12 days at 25±2°C. After incubation, the diameters of grow were measured as follows (Figure 12) (**Lourougnon *et al.*, 1991)**:

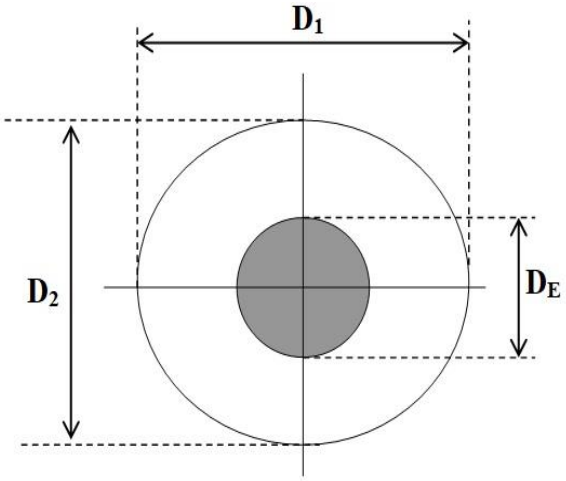

Figure 12: Measurement of the diameters of grow

$$D = \frac{D_1 + D_2}{2} - D_E$$

D_1 and **D_2**: perpendicular diameters of mycelia growth,

D_E: Diameter of mycelia disc inoculated,

D: Mean diameter of mycelia growth.

The efficiency of the essential oils was measured in terms of percentage of mycelial growth inhibition [MGI (%)] calculated using the following formula:

% I = ((Do – De)/Do) X 100 where Do is the diameter of the mycelia growth in the negative control and De the diameter of the mycelia growth in the oil supplemented plates.

Petri dishes with the lowest concentration of essential oil without any growth were recorded as the minimal inhibitory concentration (MIC).

II.3.4.2 Inhibition of fungal conidia germination

II.3.4.2.1 Preparation of conidia suspension

From 5-day-old culture plates of isolates incubated at 25±2°C, conidia were harvested by flooding PDA plates with 5 mL of physiologic liquid (0.9% of NaCl). The obtained solution was centrifugated at 2000 rpm for 2 minutes. Then, the supernatant was collected and conidia concentration was determined with the aid of a haemocytometer and adjusted to $2x10^6$ conidia/mL with physiologic solution.

II.3.4.2.2 Microdilution assay

The conidia germination assay was performed using microdilution method in 96-wells plate as described by **Toghueo *et al*., (2016)**. Briefly, 100 µL of sterilized Patato Dextrose Broth (PDB) medium supplemented with chloramphenicol and prepared according to the manufacturer's inctructions (Appendice 3) were distributed in all wells followed by addition of 100 µL of stock essential oil to the first well. After thorough mixing, a twofold serial dilution of first term 11.33 µL/mL and the last term 0.09 µL/mL was realized by successive transfer of 100 µL from the first wells into subsequent wells. Then, 100 µL of fungi conidia at $2x10^6$ spores/mL prepared in physiologic liquid (Appendice 3) were introduced in the wells excepted those of sterility control to achieved final tested charge of 10^6 spores/mL. Sterility control consisted of PDB medium without inoculation. Negative control was prepared with Tween 80 instead of essential oils while positive control wells were prepared using propiconazole. Test was performed in triplicate. Upon incubation at 25±2°C for 20 h, the microdilution plates were read on a Microplate Reader at 450 nm. The obtained value of optical density (OD) served to determine the conidia germination inhibition percentage [CGI (%)] as follow:

$$CGI\ (\%) = ((OD_c - OD_t)/ (OD_c)) \text{ X } 100$$

OD_c was the mean OD values of the negative control well and OD_t the mean OD values of the test well.

The lowest concentration given 100% of CGI (%) was the MIC.

II.4 Statistical analysis

The doses causing 50 % (KD_{50}) and 90% (KD_{90}) knockdown as well as those inducing 50% and 90% (LD_{50} and LD_{90} respectively) mortality were determined by probit analysis (**Finney, 1952**) using Online Tool (OPSTAT) (http://14.139.232.166/Probit/probitanalysis.html). The values of LD, KD and IC were considered significantly different if the 95% fiducial limits did not overlap. The differences between means were tested through the analysis of variance (ANOVA) followed by Fisher's Least Signifcant Difference test at P=0.05 level of signifcance using Statgraphics Plus 5.1 statistical package.

RESULTS AND DISCUSSION

CHAPTER III: RESULTS AND DISCUSSION

III.1 Survey on the occurrence of *Tuta absoluta* in Foumbot production basin

III.1.1 Results

III.1.1.1 Characteristics of tomato farmer's

The growers aged from 33 to 45 years old were more engaged in tomato production (57.69%). The men with 95.33% were active people compared to women representing 6.45% of tomato farmers. According to the instruction level, 8.33% of tomato farmers was not educated while 41.67% achieved primary education, 41.67% achieved secondary education and 8.33% have been at university level. Thus, 91.67% (primary, secondary and university educations) of the respondents had the ability to perceive, adopt and implement pest management practices in the field.

Agriculture is the main activity for 96.15% of Foumbot tomato growers. Besides tomato cultivation, many crops were grown including sweet pepper, eggplant, watermelon, cucumber and bean. Amongst these cultures, tomato is recorded by 89% and 11% of farmers respectively as the first and second sources of agricultural income, showing the high economic value of tomato in the area. Tomato is cultivated by 69.57% of farmers during rainy and dry seasons in arid and marshy areas respectively, while 21.74% grown in dry season and 8.69% in rainy season.

III.1.1.2 *Tuta absoluta* perception by tomato growers

III.1.1.2.1 Farmers knowledge on *T. absoluta*

In all the villages surveyed, farmers know *T. absoluta* and called it "Boko-Haram" due to it highly destructing effect as this Islamic sect known in Far-North Region of Cameroon. Table 4 revealed that this pest is more known in Mangoum (42.86% of growers) and less known in Mbantou (3.57% of growers) localities. However, 8.93% of farmers mainly in Mangoum did not have knowledges about *T. absoluta*.

Table 4: Percentage of repondents with or without knowledges on *Tuta absoluta* in Foumbot villages surveyed

	Percentage of Respondents				
	Fossett	Mangoum	Fossang	Mbantou	Mougnie
Respondents knowing *T. absoluta*	26.79	42.86	12.5	3.57	5.36
Respondents without knowledges about *T. absoluta*	0	8.93	0	0	0

III.1.1.2.2 Major constraint to tomato production

According to growers, *T. absoluta* was pointed as the major constraint to tomato production in Fosset (17.86% of farmers), Mangoum (21.43% of farmers), Fossang (7.14% of farmers) and Mougnie (3.57% of farmers) villages (Table 5). In fact, this study was carried out in dry season where high temperatures occurred and favoured optimal development of *T. absoluta* reported as one of the main insect pest of tomato.

Table 5: Constraints to tomato production according to the growers in Foumbot villages surveyed

	Percentage of tomato farmers				
Constraints	Fosset	Mangoum	Fossang	Mbantou	Mougnie
Tuta absoluta	17.86	21.43	7.14	5.36	3.57
Helicoverpa armigera	12.50	8.93	5.36	5.36	1.79
Trialeurodes vaporariorum	0.00	1.79	0.00	0.00	0.00
Ralstonia solanacearum	0.00	1.79	0.00	0.00	0.00
Fungal disease	0.00	1.79	0.00	0.00	0.00
Climate	0.00	5.36	0.00	0.00	0.00

III.1.1.2.3 Yield loss associated to *Tuta absoluta*

Yield loss associated to *T. absoluta* damages varied from 10 to 100%, two to seven days post-infestation. The mean yield loss was 61.67%. High yield loss values from 73 to 100% were recorded by 14.29%, 7.14% and 8.93% of tomato growers respectively in Fosset, Mangoum and Fossang villages (Table 6). Low yield loss values from 10 to 30% were reported by 8.93% and 10.71% of tomato growers respectively in Mangoum and Mbantou localities. This variation could be explained by the fact that farmers for whom low loss rates

were noted visited their fields daily, consulted agricultural technicians, and were members of cooperatives which advised them on control measures against *T. absoluta*. Moreover, some growers increased the frequency of application of conventional products and others used unconventional chemicals products as Petroleum and Coragen.

Table 6:Yield loss associated to *Tuta absoluta* infestation recorded by the growers in Foumbot villages surveyed

	Percentage of tomato growers				
Yield loss	Fosset	Mangoum	Fossang	Mbantou	Mougnie
(10-30)%	0.00	8.93	0.00	10.71	0.00
(31-51)%	0.00	8.93	0.00	8.93	8.93
(52-72)%	10.71	0.00	0.00	12.50	0.00
(73-100)%	14.29	7.14	8.93	0.00	0.00

III.1.1.3 Field characteristics

III.1.1.3.1 Cultivated surfaces and cropping systems

More tomato farmers (52.17%) grown fields from 1 to 8 hectares while 43.48% cultivated less than 1 hectare of area and 4.35% more than 8 hectares of surface for national marketing and to neighboring countries. Tomato is cultivated either in monoculture or in mixed cropping system in association with bean. Intercropping improves soil fertility and increases crop yield. This could be due to the nitrogen fixation effect of bean crop that improves soil fertility and encourages plant growth.

III.1.1.3.2 Cultivated varieties

In different localities studied, local (Rio grande) and hybrid (Rio Tinto F1, Rodeo 14 F1, Sakata F1, F1 Sumo) varieties were grown but in Mangoum (25% of farmers cultivated hybrid variety while 10.71% grown local variety) and Mbantou (17.85% sowed hybrid variety versus 14.29% for local variety) villages, hybrid variety is mostly preferred due to it quality and productivity (Table 7).

Table 7: Tomato variety cultivated by growers in Foumbot villages surveyed

	Percentage of tomato growers				
Variety	Fosset	Mangoum	Fossang	Mbantou	Mougnie
Local	7.14	10.71	3.57	14.29	7.14
Hybrid	7.14	25	3.57	17.85	3.57

III.1.1.3.3 Damages associated to *Tuta absoluta* damages on tomato plants

Typical damages associated to *T. absoluta* were observed at vegetative period, flowering, early fruiting and mature fruiting stage. The main damages were larvae mediated-mines under leaves cuticles, feeding with frass production, folding, chlorosis and drying of leaves, drying and dropping of flowers, holes on fruits with rot development, stunting and death of plants (Figure 13).

Figure 13: Damages of *Tuta absoluta* infestation recorded in surveyed fields

a: larvae mediated-mines, folding and drying of leaves; b: Penetration of *T. absoluta* larvae into tomato fruit; c: Severe infestation resulting in high crop loss in Mangoum village

III.1.1.3.4 Phytosanitary practices to control *T. absoluta*

Growers in Fosset (21.43%), Mangoum (37.5%), Fossang (28.57%), Mbantou (1.79%) and Mougnie (3.57%) villages used mostly conventional chemicals unauthorized on tomato plants, but applied on cotton plants. These chemicals were Coragen 20 SC, Cypercot, Emacot Fort 100 WG, Moran 30 and Caiman B 50 WG, which could negatively affect the growth and the development of tomato plants, change the nutritive quality of fruits. Conventional chemicals authorized to be applied on tomato plants as Cigogne 12 EC, Doyen 62 EC, K-optimal and Lynx were used by 3.57% and 1.79% of tomato growers respectively in Mangoum and Fossang localities. Unconventional chemicals like Petroleum were applied in the fields by 1.79% of farmers in Mangoum village (Table 8).

Table 8: Phytosanitary measures used by growers in Foumbot villages surveyed

	Percentage of tomato growers				
Control measures used	Fosset	Mangoum	Fossang	Mbantou	Mougnie
Traditionnal practices	0.00	0.00	0.00	0.00	0.00
Conventional chemicals unauthorized	21.43	37.50	28.57	1.79	3.57
Conventional chemicals authorized	0.00	3.57	1.79	0.00	0.00
Unconventional chemicals	0.00	1.79	0.00	0.00	0.00

III.1.1.5 Incidence and severity of damages associated with *T. absoluta* infestation in Foumbot tomato fields

Tuta absoluta occurred in all the sample fields in Foumbot subdivision with a global damage incidence and severity of 93.20%. and 4.40 (1-5 rating scale) respectively. The One Way Analysis of Variance (ANOVA) between sample sites with respect to the severity of damages showed significant difference between severity mean values ($p<0.05$). The lowest severity was recorded at Fosset (3.30) and the highest value at Mougnie and Mbantou (5) villages (Table 9).

Table 9: Incidence and severity of damages in different Foumbot villages surveyed

	Percentage (%)	1 to 5 rating scale
Villages	Incidence	Severity
Mangoum	82.26±35.49[a]	4.30±1.20[ab]
Fosset	93.97±2.81[a]	3.30±0.40[a]
Fossang	93.16±6.05[a]	4.50±0.40[ab]
Mougnie	96.48±5.40[a]	5.00±0.00[b]
Mbantou	100.00±0.00[a]	5.00±0.00[b]

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

II.1.1.6 Factors related to the damage indexes

II.1.1.6.1 Influence of tomato growth stage on the damage indexes

The studied fields were classified with respect to the growth stages as follows: vegetative period, flowering, early fruiting and fruit maturity. The recorded data revealed that the level of infestation was significantly different between growth stage ($p<0.05$) with less and high damage indexes respectively at flowering (incidence:8.06% and severity:1.60) and mature fruiting stages (incidence:97.61% and severity:4.90) (Table 10).

Table 10: Incidence and severity of damages as function of tomato growth stage

	Percentage (%)	1 to 5 rating scale
Tomato growth stage	Incidence (%)	Severity
Flowering	8.06±0.50[a]	1.60±0.50[a]
Early Fruiting	22.55±4.80[b]	2.50±0.80[b]
Vegetative	93.97±5.90[c]	3.30±0.50[c]
Fruit maturity	97.61±3.91[c]	4.90±0.20[d]

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

II.1.1.6.2 Influence of tomato variety on the damage indexes

The tomato fields were categorized into two classes with respect to the cultivated variety as hybrid and local. The analysis of variance indicated significance difference

($p<0.05$) between infestation degree of local (incidence:8.06% and severity:1.64) and hybrid (incidence:88.26% and severity:4.46) genotypes (Table 11).

Table 11: Incidence and severity of damages as function of cultivated tomato variety

	Percentage (%)	1 to 5 rating scale
Variety	Incidence (%)	Severity
Local	8.06 ± 3.80^{a}	1.64 ± 0.23^{a}
Hybrid	88.26 ± 6.9^{b}	4.46 ± 0.99^{b}

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

III.1.2 Discussion

Tuta absoluta was the main threat to tomato production in Foumbot villages surveyed, causing mean yield loss of 61.67% from two to seven days post-infestation. Of note, **Konje *et al*., (2019)** reported tomato borer as one of the insect which hinder tomato production in Santa subdivision, Nord-west region of Cameroon. Moreover, *T. absoluta* is reputated to devastate quickly tomato fields in a short time, above 48 hours **(Ajibade, 2017)**.

T. absoluta occurred in all fields surveyed with mean incidence and severity of 93.20% and 4.40 (1-5rating scale) respectively. Indeed, tomato is the primary host of this pest which occurred with severe infestation in favourable condition (30˚C). Similar results were highlighted with heavy infestation of *T. absoluta* in different parts of the world such as America, Europe, Africa and Asia (**Desneux *et al*., 2010; Ajibade, 2017; Rwomushana *et al*., 2019)**. *T. absoluta* invaded at all growth stage observed with high damage indexes at the fruit maturity stage (incidence: 97.61% and severity: 4.90). This result could be explained by the fact that there was enough food supply at the end of the crop cycle that promoted colonization, feeding and reproduction. **Torres *et al*., (2001)** reported that with availability of host plants, adult moth continues to oviposite, thus the insect pest density enhances and obviously the damage. In addition, **Nayana *et al*., (2018)** has shown a significant increase in the insect pest density at the end of the cropping cycle with direct effect on attack level. The damage indexes varied according to the variety with high value for hybrid genotype (incidence: 88.26% and severity: 4.46). Hybrid variety and local variety could be genetically different and Hybrid variety might have less resistance gene against insect. To limit damages

due to *T. absoluta*, synthetic chemical products were used with accumulation of residues on fruits, human and environmental toxicity. Thus, it is urgent to find ecological product such as essential oils to manage this insect pest.

III.2 Insecticidal potential of *Thymus vulgaris* and *Cymbopogon citratus* essential oils against *Tuta absoluta*

III.2.1 Results

III.2.1.1 Yield extraction and chemical profile of essential oils

Essential oils from *C. citratus* and *T. vulgaris* were obtained with respective yields of 0.20% and 0.30% (w/w) and had clear-yellow and pale-yellow colorations respectively. The GC/MS profiling of both essential oils from *T. vulgaris* and *C. citratus* indicated a total of 16 and 21 compounds respectively, representing 99.98% and 99.18% of total detected components (Table 12). Specifically, *T. vulgaris* oil was predominantly constituted of Thymol (21.53%) and α-Pinene (Dextro) (17.43%). On the other hand, Neral (34.48%) and Geranial (34.37%) were the major components found in *C. citratus* oil. Two compounds (β-myrcene and caryophyllene) were found in both essential oils, cumulatively representing 12.15% and 13.15% of the *T. vulgaris* and *C. citratus* oils respectively.

Table 12:Chemical composition of *Thymus vulgaris* and *Cymbopogon citratus* essential oils

N°	Compound Name	Retention Index	Percentage (%)	
			T. Vulgaris	*C. Citratus*
1	3-Octenol	-	3.42	-
2	Bicyclo[3.1.0]hexane, 6-methylene-	-	-	0.40
3	α-Thujene	915	3.55	-
4	α-Pinene (dextro)	920	17.43	-
5	Camphene	929	1.96	-
6	β –Myrcene	948	5.51	12.84
7	o-Cymene	969	15.37	-
8	α –Pinene	971	-	1.45
9	Durene	973	4.01	-
10	Z-Ocimene	977	-	0.78
11	cis-β-Terpineol	995	1.71	-
12	Linalool	1006	-	3.62
13	Linalool, formate	1007	5.90	-
14	Photocitral B	1015	-	0.40
15	1,5-Heptadiene, 3,3-dimethyl-, (E)-	1030	-	1.450
16	(3R)-(+)-Citronellal	1034	-	0.67
17	Camphor	1037	1.21	-
18	endo-Borneol	1048	4.56	-
19	4-Terpineol	1052	3.72	-
20	Isothymol methyl ether	1060	1.80	-
21	2-Undecanone	1082	-	2.57
22	Neral	1085	-	34.48
23	Geranial	1101	-	34.37
24	Thymol	1113	21.53	-
25	Geranic acid	1118	-	1.39
26	Caryophyllene	1122	6.64	0.31
27	Germacrene D	1137	1.66	-
28	Isoledene	1140	-	0.22
29	2-Tridecanone	1141	-	0.92
30	δ-Cadinene	1146	-	0.18
31	Safranal	1166	-	0.25
32	3,7-Dimethyl-2,6-octadien-1-ol, acetate	1195	-	1.83
33	β-Gurjunene	1173	-	0.51
34	α –Cadinol	1180	-	0.29
35	Isoamyl cinnamate	1203	-	0.25
	Total		99.98	99.18

III.2.1.2 Insecticidal effect of essential oils against *Tuta absoluta*

III.2.1.2.1 Contact toxicity assay

❖ Effects of the essential oils on *T. absoluta* larval mortality by contact route

The results obtained revealed that both essential oils had dose-dependent larvicidal effects against *T. absoluta* (Figure 14) compared to the positive and negative controls which induced no mortality at the concentration tested after 4 hours of exposure. Therefore,

continued observation of the positive control assays showed 10% larvicidal effect upon 24 hours exposure. This finding indicated that the two essential oils are fast-acting when compared to the positive control (λ-cyhalothrin 15g/L + acetamiprid 20g/L). The LD_{50} and LD_{90} values were respectively 0.33 µL/mL and 1.64 µL/mL for *C. citratus* oil and 0.61 µL/mL and 1.82 µL/mL for *T. vulgaris* oil after 4 hours of exposure (Table 13). There was no significant difference between *C. citratus* and *T. vulgaris* oils as their fiducial limits overlapped.

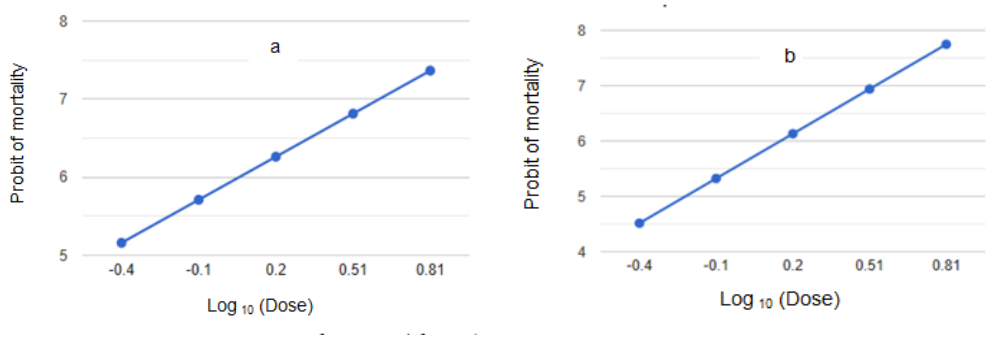

Figure 14: Variation in *Tuta absoluta* larval mortality as a function of essential oils [*Cymbopogon citratus* (a) and *Thymus vulgaris* (b)] doses by contact as per the probit transformation model

Table 13: LD50 and LD90 values of *Thymus vulgaris* and *Cymbopogon citratus* oils on fourth larval instar of *Tuta absoluta* after 4 hours of exposure by contact toxicity

Essential oil	LD_{50} (FL 95%) (µL/mL)	LD_{90} (FL 95%) (µL/ mL)
T. vulgaris	0.61 (0.32-1.16)	1.82 (0.95-3.49)
C. citratus	0.33 (0.13-1.42)	1.64 (0.63-4.27)
Lynx®	Nd	Nd
Significance	Ns	Ns

LD_{50}: Dose causing 50% mortality; FL 95%: 95% fiducial limits; LD_{90}: Dose causing 90% mortality; ns: not significant (95% fiducial limits were overlapped); ND: Not determined, positive control plates were tested at a single concentration and there was no dead up to 4 hours

❖ **Knockdown effects of the essential oils toward *T. absoluta***

Results from the knockdown assay showed that from the five doses tested (0.4; 0.8; 1.6; 3.2 to 6.4 µL/mL), only three (0.4; 0.8 and 1.6 µL/mL) effectively knocked down (immobilized but not killed) the larva (Figure 15), while at higher concentrations of 3.2 and 6.4 µL/mL, all the larva exposed to the essential oils died. The Probit model depicted a dose dependent trend with increasing percentage of larva knockdown as the dose augmented. The KD_{50} and KD_{90} values of 0.19 µL/mL and 0.78 µL/mL for *C. citratus* oil and 0.59 µL/mL and 1.09 µL/mL for *T. vulgaris* oil were obtained (Table 14). No significant difference was observed between *C. citratus* oil and *T. vulgaris* oil as their confidence limits were overlapped.

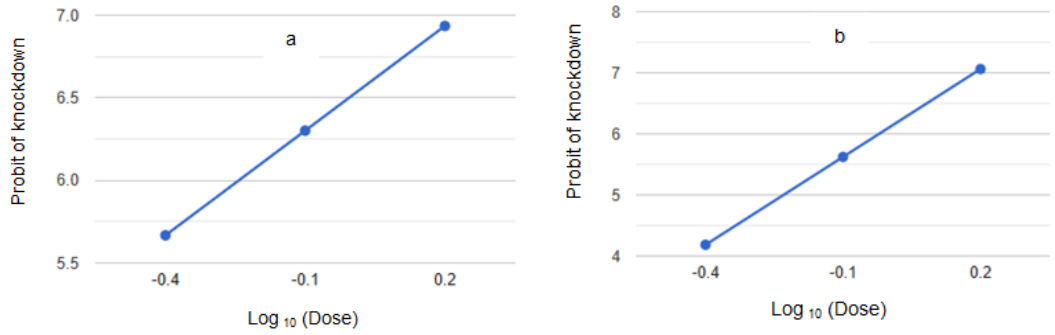

Figure 15: Variation in *Tuta absoluta* larval knockdown as a function of essential oils [*Cymbopogon citratus* (a) and *Thymus vulgaris* (b)] doses by contact as per the probit transformation model

Table 14:KD50 and KD90 values of *Thymus vulgaris* and *Cymbopogon citratus* oils on fourth larval instar of *Tuta absoluta* after 4 hours of exposure by contact toxicity

Essential oil	KD_{50} (FL 95%) (µL/mL)	KD_{90} (FL 95%) (µL/mL)
T. vulgaris	0.59 (0.09-3.55)	1.09 (0.18-6.59)
C. citratus	0.19 (0.002-14.89)	0.78 (0.01-60.49)
Lynx®	ND	ND
Significance	Ns	Ns

KD_{50}: Dose causing 50% knockdown; FL 95%: 95% fiducial limits; KD_{90}: Dose causing 90% knockdown; ns: not significant (95% fiducial limits were overlapped); ND: Not determined, positive control plates were tested at a single concentration and there was only 5% knockdown at 4 hours

❖ **Post-treatment rearing of surviving larva**

Post-treatment rearing of surviving larva revealed that at 0.4 µL/mL, *T. vulgaris* oil could significantly increase the larval instar duration by 3 days when compared with *C. citratus* oil (2 days 14 hours) and the negative control (2 days 16 hours), but showed statistically (LSD) equal effect compare to the reference insecticide (λ-cyhalothrin 15g/L + acetamiprid 20g/L) (2 days 18 hours) (Figure 16). Estimation of pupal duration revealed that both essential oils lengthened the transition time from pupae to adult when compared to the reference insecticide. *T. vulgaris* and *C. citratus* oils prolonged the pupal duration by 7 days 5 hours and 7 days respectively while the positive control elicited only 6 days 11 hours duration.

At 0.8 µL/mL, elicited larval duration for *C. citratus* oil (3 days) and the positive control (2 days 18 hours) was not significantly different to the negative control (2 days 16 hours). Conversely, *T. vulgaris* oil significantly (LSD, $p < 0.05$) lengthened larval period by 3 days 16 hours, more than the reference insecticide (2 days 18 hours). Meanwhile, no significant difference was observed on pupal duration between the effects of *T. vulgaris* oil (6 days 16 hours) and the control treatments (6 days 17 hours for Tween 80 and 6 days 11 hours for λ-cyhalothrin 15g/L + acetamiprid 20g/L). Whereas, exposure to *C. citratus* oil lengthened pupal duration by 8 days when compared to the other treatments (LSD, $p < 0.001$).

Of note, contact larvicidal assay led to the death of all surviving larva above the concentration of 0.80 µL/mL as evidenced in Figure 13 below

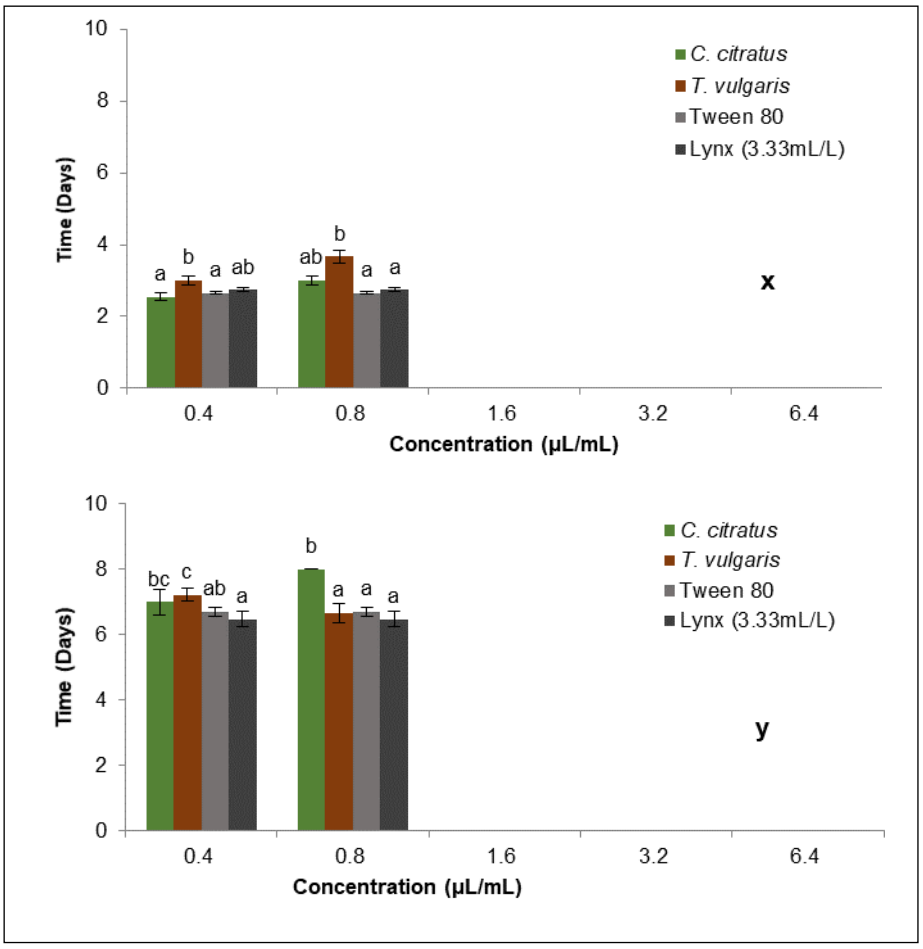

Figure 16: *Tuta absoluta* larval (x) and pupal (y) duration through essential oils by contact toxicity

Bars bearing different letters depict significant difference as referred to the Least Significant Difference (LSD) test. At concentrations above 0.8 µL/mL, all the larva and pupa died

III.2.1.2.2 Fumigant toxicity

❖ **Effects of the essential oils on *T. absoluta* larval mortality by fumigation route**

Both essential oils from *T. vulgaris* and *C. citratus* exhibited larvicidal activity through fumigation (Figure 17). The mortality rate increased in a dose-dependent manner. No mortality was observed in negative and positive controls after 4 hours. It is noteworthy that further observation of the positive control plates for 24 hours led to no larvicidal effect contrarily to the outcome in the contact toxicity assay. This result further confirmed the fast-acting profile of the essential oils. The LD_{50} and LD_{90} values of 1.48 and 2.85 µL/mL for *C. citratus* oil and 3.05 and 7.61 µL/mL for *T. vulgaris* oil were obtained after 4 hours of exposure (Table 15). A comparative analysis of the LD_{50} values clearly indicated a

statistically similar larvicidal effect of *T. vulgaris* oil and *C. citratus* oil as their fiducial limit were overlapped.

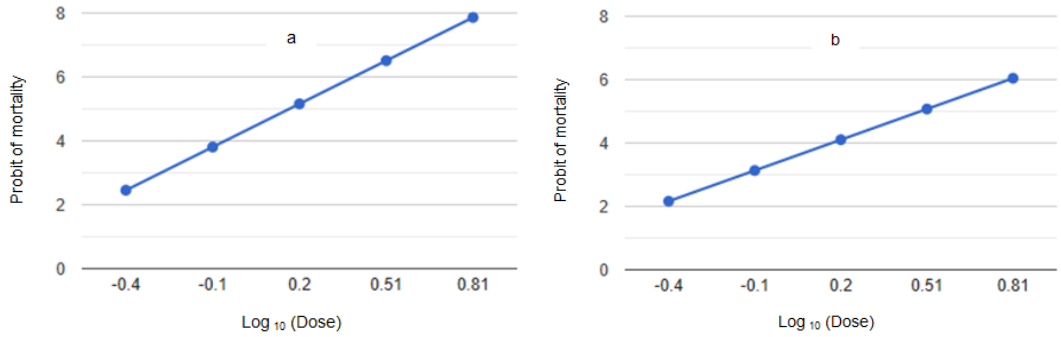

Figure 17: Variation in *Tuta absoluta* larval mortality as a function of essential oils [*Cymbopogon citratus* (a) and *Thymus vulgaris* (b)] doses by fumigation as per the probit transformation model

Table 15: LD50 and LD90 values of *Cymbopogon citratus* and *Thymus vulgaris* oils on fourth larval instar of *Tuta absoluta* after 4 hours of exposure by fumigation

Essential oil	LD_{50} (FL 95%) (µL/mL)	LD_{90} (FL 95%) (µL/mL)
T. vulgaris	3.05 (1.76-5.27)	7.61 (4.39-13.17)
C. citratus	1.48 (0.95-2.31)	2.85 (1.83-4.46)
Lynx®	ND	ND
Significance	Ns	Ns

LD_{50}: Dose causing 50% mortality; FL 95%: 95% fiducial limits; LD_{90}: Dose causing 90% mortality; ns: not significant (95% fiducial limits were overlapped); ND: Not determined, positive control plates were tested at a single concentration and there was no dead up to 4 hours

- **Knockdown effects of the essential oils against *T. absoluta* through fumigation route**

The immobilization of *T. absoluta* larva in response to exposure to essential oils through fumigation was calculated. Results showed on Figure 18 indicate that the larva were knocked down at the doses of 0.4; 0.8; 1.6 and 3.2 µL/mL. At 6.4 µL/mL, all the larva exposed to the essential oils died. The Knockdown percentage positively correlated with essential oil concentrations. The KD_{50} and KD_{90} values of 1.29 µL/mL and 2.69 µL/mL for *C. citratus* oil and 2.57 µL/mL and 5.22 µL/mL for *T. vulgaris* oil were recorded after 4 hours of exposure

(Table 16). Based on their fiducial limits which are overlapped, *C. citratus* and *T. vulgaris* oils exerted similar knockdown effect on *T. absoluta* larvae.

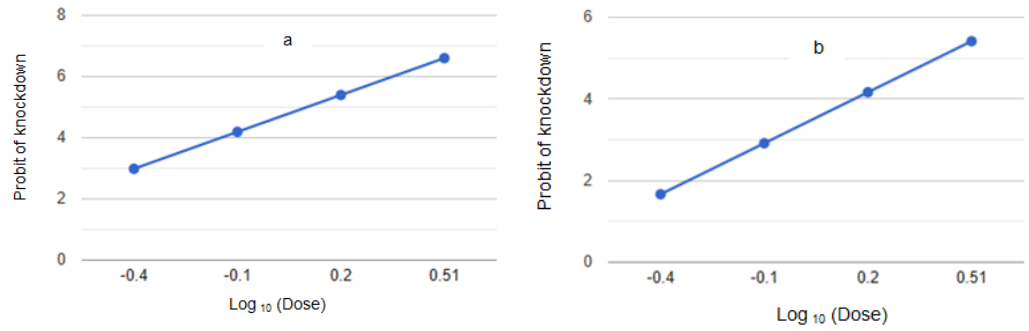

Figure 18: Variation in *Tuta absoluta* larval knockdown as a function of essential oils [*Cymbopogon citratus* (a) and *Thymus vulgaris* (b)] doses by fumigation as per the probit transformation model

Table 16: KD50 and KD90 values of *Thymus vulgaris* and *Cymbopogon citratus* oils on fourth larval instar of *Tuta absoluta* after 4 hours of exposure by fumigation

Essential oil	KD_{50} (FL 95%) (µL/mL)	KD_{90} (FL 95%) (µL/mL)
T. vulgaris	2.57 (1.29-5.10)	5.22 (2.62-10.39)
C. citratus	1.29 (0.68-2.46)	2.69 (1.41-5.15)
Lynx®	ND	ND
Significance	Ns	Ns

$KD_{50:}$ Dose causing 50% knockdown; FL 95%: 95% fiducial limits; $KD_{90:}$ Dose causing 90% knockdown; ns: not significant (95% fiducial limits were overlapped); ND: Not determined, positive control plates were tested at a single concentration and there was no knockdown at 4 hours

❖ **Post-treatment rearing of surviving larva**

At the concentration of 0.4 µL/mL, all the tested essential oils significantly decreased the larval and pupal durations as compared to the controls (Figure 19). Thus, *T. vulgaris* and *C. citratus* oils respectively induced a duration of larvae by 1 day 5 hours and 1 day 6 hours, and the pupal duration by 3 days 20 hours and 4 days 11 hours. The positive (λ-cyhalothrin 15g/L + acetamiprid 20g/L) and negative (Tween 80) controls elicited enhanced larval durations of 2 days 9 hours and 2 days 6 hours respectively; and pupal durations were 7 days 9 hours and 7

days respectively. Overall, the essential oils showed less potent effects as compared to the controls.

At the concentration of 0.8 µL/mL, although no significant difference was recorded for elicited larval instar duration upon exposure to *T. vulgaris* oil and the controls (2 days 10 hours for *T. vulgaris* oil versus 2 days 9 hours and 2 days 6 hours for Lynx and Tween 80 respectively), *C. citratus* oil more significantly augmented the larval duration ($p < 0.001$) relatively to the negative control (2 days 14 hours for *C. citratus* oil and 2 days 6 hours for Tween 80). Of note, *C. citratus* oil (2 days 14 hours) and *T. vulgaris* oil (2 days 10 hours) exerted statistically similar effects. Both essential oils significantly lengthened pupal duration with 7 days 18 hours for *T. vulgaris* oil and 8 days 23 hours for *C. citratus* oil, compared to 7 days and 7 days 9 hours for the negative and positive controls respectively.

At 1.6 µL/mL, all the tested essential oils significantly ($p < 0.001$) prolonged both larval and pupal durations compared to controls. Larval duration was 3 days 7 hours and 3 days respectively for *T. vulgaris* oil and *C. citratus* oil while the positive and negative controls elicited 2 days 9 hours and 2 days 6 hours respectively. The recorded pupal duration for *T. vulgaris* and *C. citratus* oils was 7 days 21 hours and 9 days respectively while the positive and negative controls induced 7 days 9 hours and 7 days duration respectively. *C. citratus* oil exerted highly potent effect on pupal duration relative to *T. vulgaris* oil and the reference insecticide. At concentrations above 1.6 µL/mL, there was no transition from larvae to the pupae stage

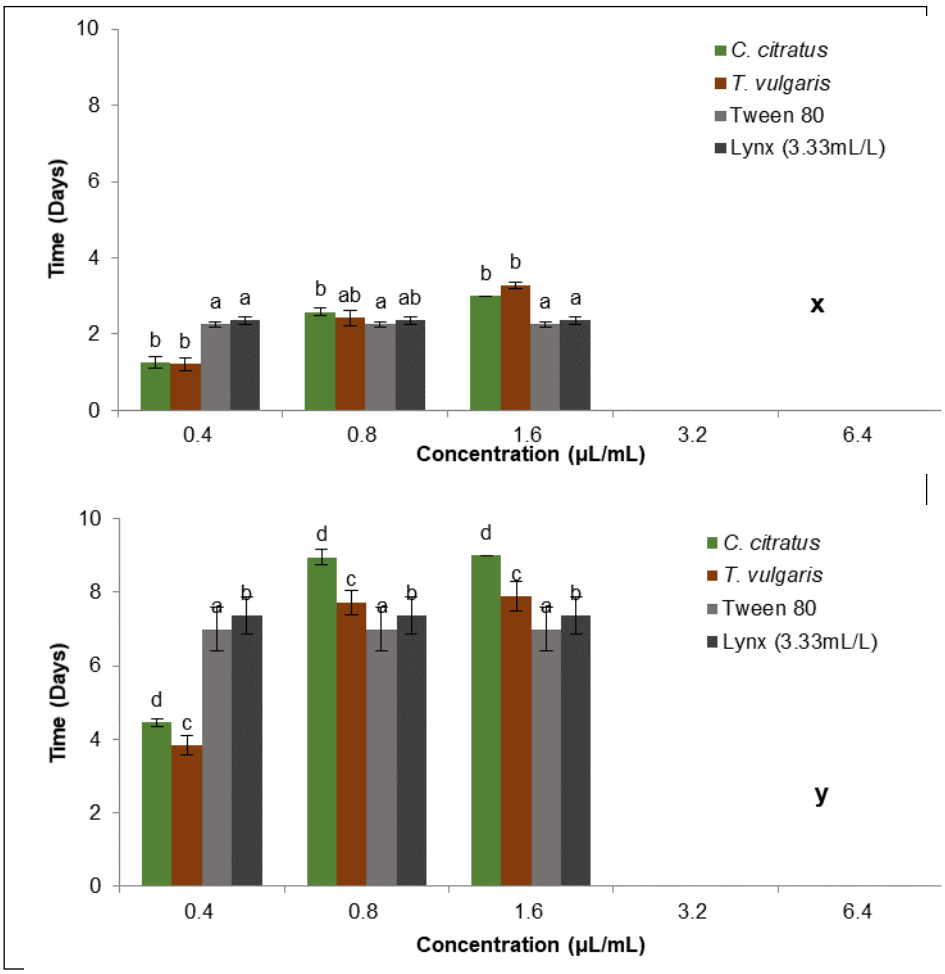

Figure 19: *Tuta absoluta* larval (x) and pupal (y) durations through essential oils by fumigation

Bars bearing different letters depict significant difference as referred to the Least Significant Difference (LSD) test. At concentrations above 1.6 µL/mL, all the larva and pupa died

III.2.1.3 Mecanism of action of essential oils: anti-acetylcholinesterase activity

Thymus vulgaris and *C. citratus* EOs significatively ($p < 0.05$) inhibited AChE in a dose-dependent manner (Figure 20). At the lowest concentration (0.4µL/mL), the percentages of inhibition were 13.01% and 26.76% respectively for *T. vulgaris* and *C. citratus* oils and were 99% and 74.99% at the highest concentration (6.4µL/mL). The IC_{50} of the tested EOs were given in Table 17. Statistically, both oils had similar inhibitory effect on AChE (95% fiducial limits were overlapped), with IC_{50} of 2.57 and 3.06 µL/mL for *T. vulgaris* and *C. citratus* oils respectively. EOs were less efficient when compared with the positive control as the IC_{50} value of Lynx® was less than 0.0008 µL/mL.

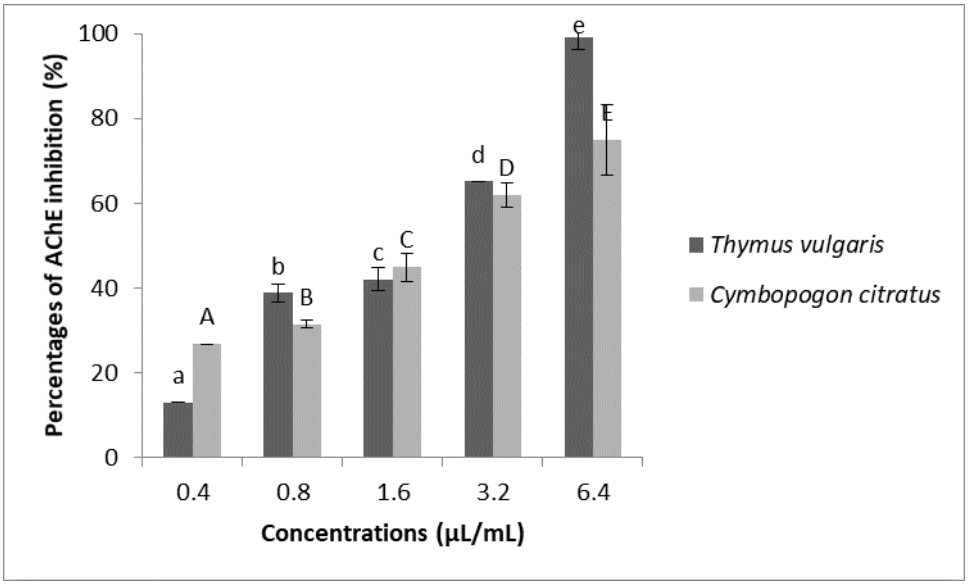

Figure 20: Inhibition percentage of acetylcholinesterase as a function of concentrations of *Cymbopogon citratus* and *Thymus vulgaris* essential oils

Bars bearing diferent letters depict signifcant diference (***p***<0.05**)** as referred to the Least Signifcant Diference (LSD) test.

Table 17: IC50 values of *Thymus vulgaris* and *Cymbopogon citratus*

Essential oil	IC_{50} (FL 95%) (µL/mL)
T. vulgaris	2.57 (0.52-8.48)
C. citratus	3.06 (0.35-3.22)
Lynx®	< 0.0008

IC_{50}: Concentration inhibiting 50% of AChE; FL 95%: 95% fiducial limits.

The effect of tested EOs on the specific activity of AChE is illustrated in Figure 21. Globally, the specific activity of enzyme treated with *T. vulgaris* and *C. citratus* oils significantly decreased ($p < 0.05$) with the concentration. At the lowest concentration (0.4µL/mL), the specific activity of AChE were $4.70x10^{-9}$ and $3.88x10^{-9}$ ΔOD/min/µg protein for *T. vulgaris* and *C. citratus* oils respectively and at the highest dose (6.4µL/mL), the activity were $0.06x10^{-9}$ and $1.44\ x10^{-9}$ ΔOD/min/µg protein respectively.

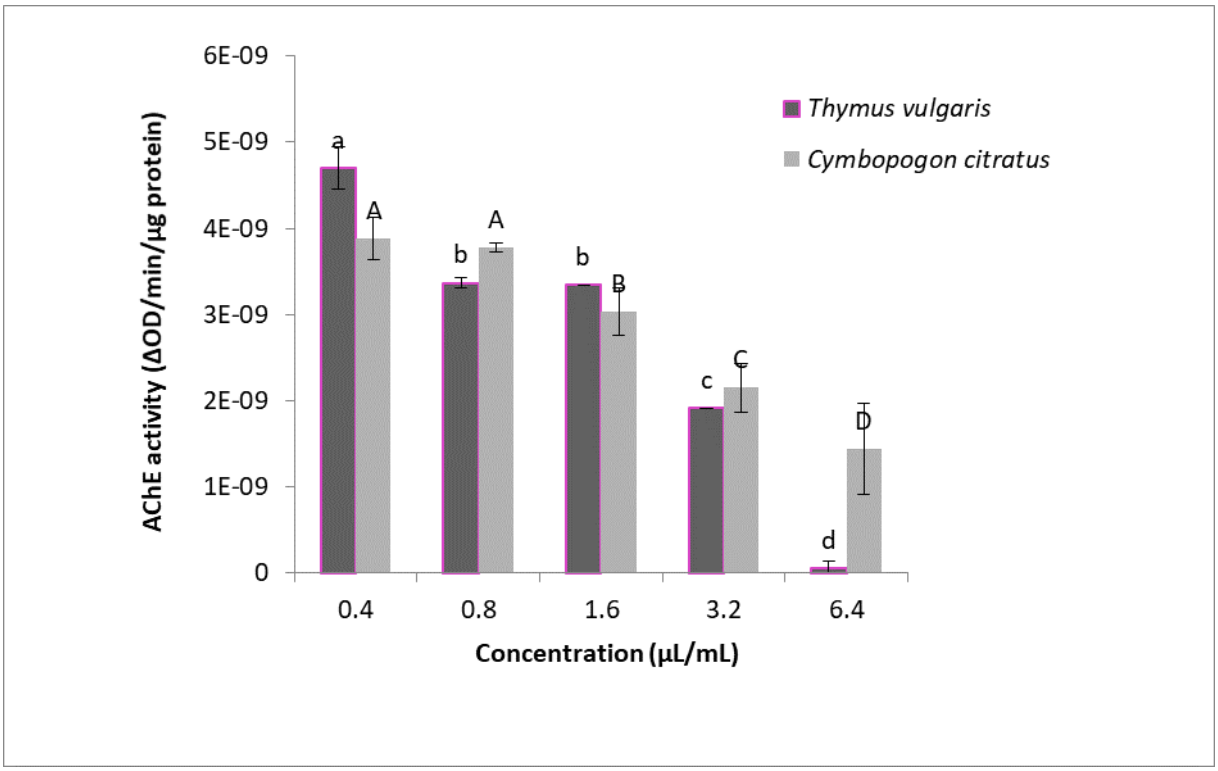

Figure 21: Specific activity of acetylcholinesterase as a function of concentrations of *Cymbopogon citratus* and *Thymus vulgaris* essential oils

Bars bearing diferent letters depict signifcant diference (***p***<0.05**)** as referred to the Least Signifcant Diference (LSD) test.

III.2.2 Discussion

The yields of extraction of *C. citratus* and *T. vulgaris* EOs in this study were 0.20% and 0.30% respectively. **Simo *et al.*, (2015)** previously reported similar yield (0.32%) from *T. vulgaris* essential oil. Differently, **Nguefack *et al.*, (2009)** reported higher extraction yields of 0.65% and 0.70% from *T. vulgaris* and *C. citratus* dried materials respectively. Many factors like the soil type, harvest period, plant age, growing conditions and plant material status (dried or fresh) have been reported to affect the yield of essential oils (**Aminzadeh *et al.*, 2010**; **Benbelaid *et al.*, 2013**). The chemical analysis of the essential oils revealed Thymol (21.53%) and α-Pinene (Dextro) (17.43%) as the major constituents of the *T. vulgaris* oil while, Neral (34.48%) and Geranial (34.37%) were found to be prominent in the *C. citratus* oil. Overall, 16 and 21 compounds were identified in *T. vulgaris* and *C. citratus* oils, representing 99.98% and 99.18% respectively of the overall components. Earlier findings by **Nguefack *et al.*, (2009)** and **Simo *et al.*, (2015)** indicated Thymol and p-Cymene as the major

components of *T. vulgaris* oil whereas, **Tchinda *et al.*, (2009)** reported Geranial (32.20%), Myrcene (27%) and Neral (25.70%) as the main constituents of *C. citratus* oil. Such discrepancies in chemical compositions of essential oils have been ascribed to the harvest season, plant age and soil type which can significantly impact on plant metabolism thus, on essential oil biosynthesis **(Braga *et al.*, 2005)**.

Cymbopogon citratus and *Thymus vulgaris* oils exerted prominent insecticidal effects through fumigation and direct contact toxicity routes. The mortality rate of *T. absoluta* increased in a dose-dependent manner. Previous studies reported the insecticidal activity of *C. citratus* oil by contact and fumigation on cabbage looper (*Trichoplusia ni*) (**Tak *et al.*, 2015**). Moreover, **Sammour *et al.*, (2018)** showed the larvicidal activity of formulated *T. vulgaris* oil at 0.078 % against *T. absoluta* through ingestion route, corroborating our findings to an extent. The depicted bioactivities could be also ascribed to the multitude of components of the essential oils and their interactions (**Nana *et al.*, 2015**; **Scalerandi *et al.*, 2018**). Indeed, the effect of terpenoids (that are predominent in the essential oils described in this study) on insect pest elicits neurotoxicity, reduction of growth, reproductive capability, suppression of appetite and death of the insect by starvation (**Viegas-Junior, 2003**; **Szczepanik *et al.*, 2012**). Citral (neral + geranial) that represents a huge proportion of the *C. citratus* oil (68.85%) has been reported to exhibit insecticidal properties against insect pests including, but not limited to *Ulomoides dermestoides* (**Plata-Rueda *et al.*, 2020**), *Helicoverpa armigera* and *Spodoptera litura* (**Kaur *et al.*, 2019**). It is therefore arguable that citral has a great contribution toward the recorded insecticidal potency of the *C. citratus* oil. This statement corroborates earlier emphasis by **Tak and Isman (2016)**, who reported that citral stimulates the reduction of frass production, elicits feeding deterrence and inhibits the larval growth of cabbage looper (*Trichoplusia ni*). Also, the insecticidal activity of *Thymus vulgaris* essential oil, thymol and carvacrol was previously shown against different larval stages of lesser mealworm (*Alphitobius diaperinus* Panzer, Coleoptera, Tenebrionidae) (**Szczepanik *et al.*, 2012**). The authors of this study concluded that thymol (the most abundant component in the *T. vulgaris* oil tested in this study) and carvacrol are strong deterrent and growth inhibitors, and were more active against *A. diaperinus* larvae than *T. vulgaris* oil, and could be applied as effective control for this pest. Therefore, it is relevant to consider thymol as one of the main elicitors of the insecticidal activity observed. Similarly, the insecticidal activity of α-Pinene (the second most abundant constituent of *T. vulgaris* oil) and 3-Carene on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) was previously reported by contact toxicity

and fumigation by **Langsi *et al.*, (2020)**. The high activity of α-pinene and 3-carene was suggested to be due to their ability to penetrate the insects' respiratory system (**Kim *et al.*, 2010**; **Morteza *et al.*, 2016**). They concluded that both monoterpenes were very promising and effective and could be exploited as novel phytoinsecticides against maize weevil. The cumulative effect of the described major components of *T. vulgaris* and *C. citratus* oils when added to the activities of the other essential oils' constituents represent a strong basis for justifying the elicited insecticidal and knockdown effects.

Results from this study also revealed that the studied essential oils could impact the transition of *T. absoluta* from one developmental stage to another (larvae, pupae and adult). Indeed, at 0.4 µL/mL, *T. vulgaris* oil lengthened larval period through contact toxicity meanwhile by fumigation route, it did not prolonged larval time. The results obtained further strengthened the inference of application route on the efficacy of essential oils against plant pests. Indeed, both oils, when tested through fumigation route lengthened larval and pupal duration. Through contact toxicity, results showed that *T. vulgaris* oil delayed larval and pupal stages while *C. citratus* oil prolonged pupal duration only. Overall, contact route appreared to be more efficient as this method required less values of KD_{50} and LD_{50} compared to fumigation. *C. citratus* oil is better than *T. vulgaris* oil since the first prolonged more the duration of life cycle compared to the second. On another hand, previous findings by **El-Mesallamy *et al.*, (2015)** showed that basil oil prolonged live cycle of spiny bollworm. The delay of the development cycle of the insect could be due to the fact that essential oils impair the appetite of the pest, with consequent growth retardation. Indeed, **Tak and Isman (2016)** reported that the delay of insect growth is due to the reduction of food intake. This is corroborated by the observed reduction of feeding associated with significant prolongation of life cycle rate of *Spodoptera frugiperda* by **Sosa *et al.*, (2019)**.

The study of the mechanism of action of essential oils revealed that both oils exerted an anti-acetylcholinesterase effect, resulting in a permanent stimulation, hyperexcitability and paralysis leading to death (**Begum *et al.*, 2011; Lionetto *et al.*, 2013; Rajashekar *et al.*, 2014**). Similar results were reported by **Picollo *et al.*, (2008)** which argued that EOs exerted insecticidal action through the inhibition of AChE. The capacity of studied EOs to impaid AChE activity could be ascribed to the chemical composition. Indeed, previous works reported the acetylcholinesterase inhibitory properties of monoterpenes such as citral, thymol, linalool and camphor which could act in synergism since the inhibitory activity of individual terpenes was lower than the whole oil (**Keane and Ryan, 1999**; **Jukic *et al.*, 2007; Lopez**

and Pascual-Villalobos, 2010). This part revealed that *C. citratus* and *T. vulgaris* oils exerted similar larvicidal effect by direct contact and fumigation toxicities on *T. absoluta*. Amongst damages due to *T. absoluta*, there is tomato fruit perforation which constitute routes for fungal infection as *G. candidum*. Then, the study of the efficacy of the both EOs on *G. candidum* are necessary to optimize the control.

III. 3 Antifungal potential of *Thymus vulgaris* and *Cymbopogon citratus* essential oils against *Geotrichum candidum*

III. 3.1 Results

III. 3.1.1 Isolation of *Geotrichum candidum* and pathogenicity test

From fruit infested by *T. absoluta* and presenting rot symptoms, 25 strains of *G. candidum* were isolated. The pathogenicity test revealed that only 3 were pathogenics and successfully developped sour rot symptom on fruits compared to the control (Figure 22). The disease incidence was 100% for each pathogen. Symptoms were charactarized by water-soak of fruits that contain white mycelium. As decay progressed, fruits became soft with a distinctive sour odor. In addition, the measurement of the lesion's size showed significant difference ($p<0.05$) between isolates as far as the rot diameter is concerned, the lowest and highest values account for isolates G1 (14.38 mm) and G2 (58.33 mm) (Table 18). The evaluation of rot degree revealed that all three isolates were high virulent as rot area were more than 21 mm^2 (**Chehri, 2015**).

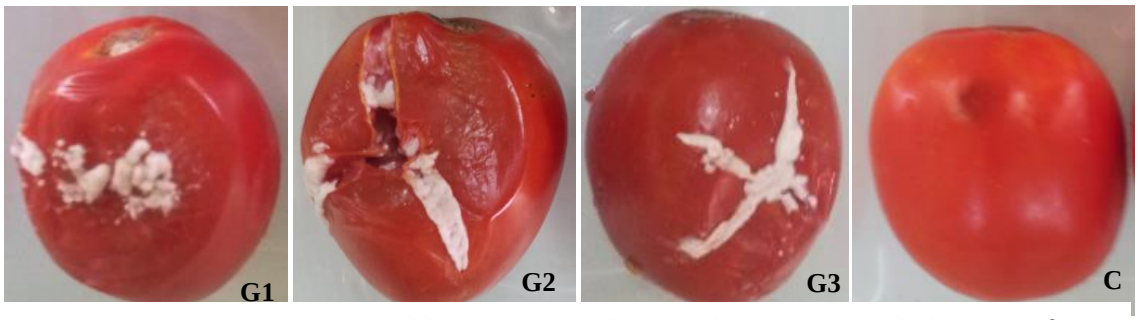

Figure 22: Rot symptoms caused by G1, G2 and G3 isolates on wounded tomato fruits (cv.Rio grande) compared to control (C)

Table 18: Rot diameter and virulence of isolates after five days of incubation

Isolate	Rot diameter (mm)	Rotted area (mm^2)	Virulence (a-d scale)	Virulence status
G1	14.38 ±1.08[a]	162.91 ±24.98[a]	D	High virulent
G2	58.33 ±2.95[b]	2677.10±267.48[b]	D	High virulent
G3	27.29± 0.36[c]	585.06±15.4[c]	D	Highvirulent

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

III. 3.1.2 Identification of *Geotrichum candidum*

III.3.1.2.1 Morphological identification

The three isolates pathogens morphologically belong to *Geotrichum* sp. based on colony morphology on PDA with milky white colony, cream reverse colour, conidiogenesis belong to arthric mode with hyaline hyphae, septate, differentiated into disarticulated hyphae, then into rectangular cells called arthric conidia. The pathogen G1 was characterized by soft and farinose aspect, hairless, diameter of colony growth was 90 mm after 10 days of culture with a predominance of vegetatif hyphae and few arthroconidia; the pathogen G2 shown hairy and roughly aspects, diameter of colony growth was 52 mm. Microscopic observation revealed abundant arthroconidia and few vegetatif hyphae and the last pathogen, G3 presented radiating pattern from the source, hairless, flat and smooth aspects, the colony growth diameter was 90 mm after 10 days of culture on PDA medium, there was a proportionality between vegetatif hyphae and arthroconidia (Figure 23). The three pathogens shown hyaline hyphae, with wide vary from 8 to 11 µm for main branches which expand branching hyphae. On the basis of these characteristics, the all three pathogens G1, G2 and G3 were attributed to *Geotrichum candidum.*

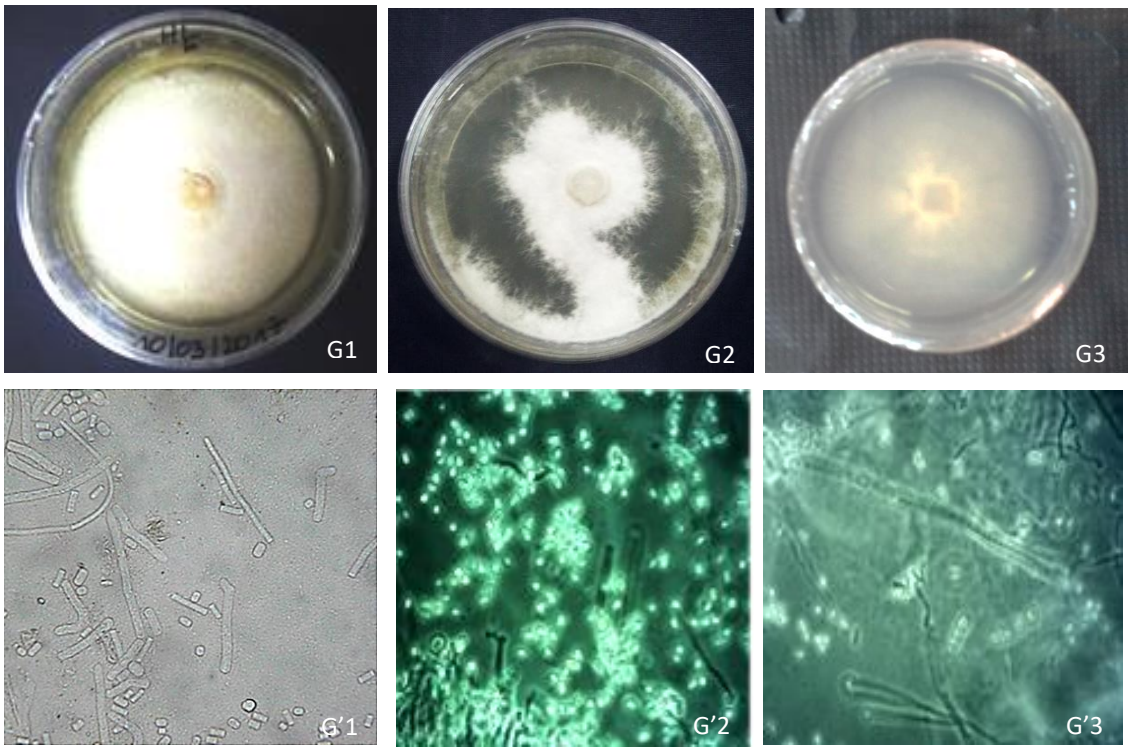

Figure 23: Macroscopy (G1, G2 and G3) and microscopy (G'1, G'2 and G'3) characterizations of the three pathogens on PDA medium

III. 3.1. 2.2 Molecular identification

- **Sequences analysis**

The sequencing of the ITS1-5.8S rDNA-ITS4 region revealed that the identity of the pathogens earlier presumed to be *Geotrichum candidum* upon morphological identification was confirmed. Similarity with sequences earlier submitted to genbank database was established. Thus, *Geotrichum candidum* G1 under accession number MK436202.1 was homologous to *Geotrichum candidum* isolate GC333-ITS1F at 98 %. While *Geotrichum candidum* G2 with accession number of MN244388.1 show 99% homology with *Geotrichum candidum* strain JYC546. *Geotrichum candidum* G3 under accession number of KY103454.1 was homologous to *Geotrichum candidum* culture CBS:11616 at 98% (Table 19).

Table 19:Phylogenetic affiliation of pathogens

Pathogens	Homologous fungi	Percentage homology	Accession number GenBank
Geotrichum candidum G1	*Geotrichum candidum* isolate GC333-ITS1F	98%	MK436202.1
Geotrichum candidum G2	*Geotrichum candidum* strain JYC546	99%	MN244388.1
Geotrichum candidum G3	*Geotrichum candidum* culture CBS:11616	98%	KY103454.1

- **Phylogenetic analysis**

Phylogenetic tree was drawn from sequences obtained through amplification using ITS1-5.8S rDNA-ITS4 region of gene (Figure 24).

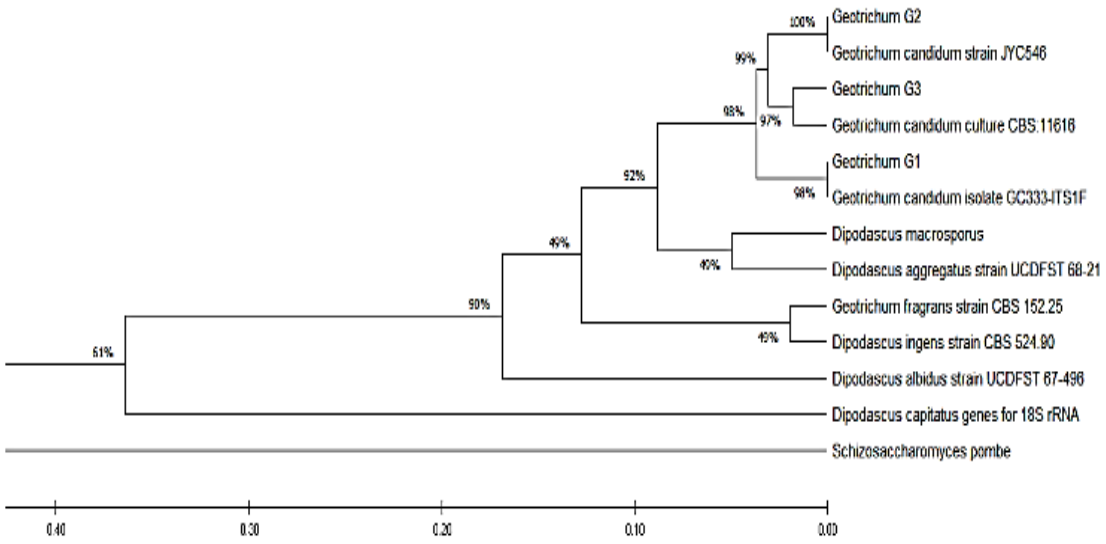

Figure 24: Phylogenetic tree based on comparison of ITS1-5.8s-ITS4 sequences of pathogenics *Geotrichum candidum* to the references strains avalailable in NCBI genbank

III. 3.1.4 Antifungal activity of *Thymus vulgaris* and *Cymbopogon citratus* essential oils against *Geotrichum candidum*

III. 3.1.4.1 Mycelia growth inhibition assay of essential oils against *Geotrichum candidum*

The *in vitro* assay showed that *C. citratus* and *T. vulgaris* essential oils significantly ($P < 0.05$) inhibited the mycelia growth of *G. candidum G1, G2 and G3* in a dose-dependent manner (Table 20 and table 21 respectively). At the lowest concentration, 16.23 %; 6.36 % and 4.83% mycelia growth inhibition percentage were obtained respectively on *G. candidum G1, G2 and G3* with *C. citratus* oil, while *T. vulgaris* oil exerted 30.14%; 49.80% and 71.59% MGI on *G. candidum* G1; *G. candidum* G2 and *G. candidum* G3 respectively. No growth occurred on the culture medium when concentrations reached 0.71 µL/mL and 5.67 µL/mL respectively for *C. citratus* oil and *T. vulgaris* oil on all the pathogens. In Propiconazole Petri dishes, no grow was observed for all the fungi until 0.02 µL/mL, which was the MIC. Statistical analysis revealed that the MIC value of *C. citratus* oil for all pathogens (0.71 µL/mL) was significantly inferior ($P < 0.05$) to the MIC value of *T. vulgaris* oil (5.67 µL/mL) for the three fungi. Then, *C. citratus* oil was more effective compared with *T. vulgaris* oil on mycelia growth of fungi.

Table 20: Mycelia growth inhibition percentage of *Cymbopogon citratus* essential oil against *Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3

	Inhibition percentage (%)		
Concentrations (µL/mL)	*G. candidum* G1	*G. candidum* G2	*G. candidum* G3
0.09	16.23±4.94[a]	6.36±2.11[a]	4.82±2.74[a]
0.18	53.07±3.74[b]	41.23±3.04[b]	20.61±2.01[b]
0.35	64.29±0.00[c]	63.49±2.75[c]	47.22±7.56[c]
0.71	100±0[d]	100±0[d]	100±0[d]
1.42	100±0[d]	100±0[d]	100±0[d]

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

Table 21: Mycelia growth inhibition percentage of *Thymus vugaris* essential oil against *Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3

	Inhibition percentage (%)		
Concentrations (µL/mL)	*G. candidum* G1	*G. candidum* G2	*G. candidum* G3
0.35	30.14±0.93[a]	49.80±6.79[a]	71.59±1.97[a]
0.71	87.40±0.17[b]	56.47±0.00[b]	90.91±1.74[b]
1.42	94.27±0.08[c]	88.63±0.68[c]	93.18±1.97[c]
2.83	94.66±1.28[c]	94.12±2.04[d]	95.45±0.00[d]
5.67	100±0[d]	100±0[e]	100±0[e]

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

III. 3.1.4.2 Conidia germination inhibition assay of essential oils against *Geotrichum candidum*

The percentage of inhibition significantly ($p < 0.05$) increased with the concentrations for the both oils (Table 22 and table 23). At 0.09 µL/mL, inhibition percentages were 65.09, 62.69 and 80% respectively on *G. candidum G1, G2 and G3* for *C. citratus* oil and were 50.10, 58.64 and 46% respectively on *G. candidum G1, G2 and G3* for *T. vulgaris* oil. The MIC values of *C. citratus* oil were 5.67, 1.42 and 0.71 µL/mL respectively for *G. candidum G1, G2 and G3*. There was significantly difference ($P < 0.05$) between these values and *G. candidum* G3 was the most sensitive to *C. citratus* oil. The MIC value of *T. vulgaris* oil was undetermined as conidia germination occurred at the highest concentration (11.33 µL/mL). Compared to *C. citratus* oil, *T. vulgaris* oil was not efficient on the conidia germination of the three fungi. The positive control, Propiconazole Tilt 25 EC totally suppressed the conidia germination of fungi at all the concentrations tested then, the MIC was undetermined.

Table 22: Conidia germination inhibition percentage of *Cymbopogon citratus* essential oil against *Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3

	Inhibition percentage (%)		
Concentrations (µL/mL)	*G. candidum* G1	*G. candidum* G2	*G. candidum* G3
0.09	65.09±0.00 [a]	62.69±1.75[a]	80±0[a]
0.18	67.76±7.56[b]	85.63±5.35[b]	92.35±0.00[b]
0.35	80.51±1.46[c]	88.50±1.74[c]	99.33±1.74[c]
0.71	92.02±0.00[d]	99.81±0.00[d]	100±0[c]
1.42	91.91±0.00[d]	100±0[d]	100±0[c]
2.83	93.85±0.00[e]	100±0[d]	100±0[c]
5.67	100±0[f]	100±0[d]	100±0[c]

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

Table 23: Conidia germination inhibition percentage of *Thymus vugaris* essential oil against *Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3

	Inhibition percentage (%)		
Concentrations (µL/mL)	*G. candidum* G1	*G. candidum* G2	*G. candidum* G3
0.09	50.10±1.40[a]	58.64±0.00[a]	46.00±1.73[a]
0.18	55.29±0.00[b]	65.13±6.39[b]	66.75±1.64[b]
0.35	69.72±0.18[c]	91.36±0.42[c]	67.00±2.03[b]
0.71	94.99±3.11 [d]	91.97±0.12[c]	70.72±0.31[c]
1.42	94.07±0.08[e]	93.03±0.00[d]	78.38±2.61[d]
2.83	94.97±0.00 [d e]	93.19±1.74[d]	87.29±2.46[e]
5.67	90.08±0.00[f]	92.81±0.07[d]	87.79±0.00[e]
11.33	93.27±0.00[g]	92.78±0.07[d]	89.88±0.84[f]

Means followed by the different letter in the same column are significantly different (***p***<0.05**)** as referred to the Least Significant Difference (LSD) test

III. 3.2 Discussion

Geotrichum candidum G1, G2 and G3 were isolated from sour rot of decaying fruits of tomato infested by *T. absoluta*. In fact, sour rot of tomatoes caused by *G. candidum* occurs in the field and postharvest settings regularly (**Fiedler, 2014**). Moreover, **Moline, (1984)** reported *G. candidum* as a wound pathogen requiring injury to enter and cause sour rot of vegetables and fruits. **Bourret *et al.*, (2013)** identified *G. candidum* as opportunistic fungi

causing rot on tomato fruits collected in an home garden of Washingston state. In addition, this pathogen has been associated to sour rot of tomato fruit grown in a commercial tomato field of the Eastern Shore of Virginia (**Fiedler, 2014**). Natural products as essential oils are recorded as sustainable alternative for phytopathogens control. Results of this study showed the effectiveness of *C. citratus* and *T. vulgaris* essential oils on the mycelia growth of the pathogens. This activity could be link to the major compounds (citral and thymol respectively) and to the interactions with others components of EOs. In this sense, **Zhou *et al.*, (2014)** reported the antifungal activity and the potential antifungal mechanisms of three volatile compounds, citral, octanal, and alpha-terpineol against *Geotrichum citri-aurantii*, one of the main postharvest pathogens of citrus. The results achieved by these authors indicated that the volatile compounds exhibited strong antifungal activity against the targeted pathogen, with MIC of 0.50 µL/mL for citral, 0.50 µL/mL for octanal, and 2.00 µL/mL for alpha-terpineol. The mechanism of action of citral, octanal, and *a*-terpineol was said to be through increasing membrane permeability causing disruption of cell membrane integrity and cell constituent release. Moreover, citral induced a decrease in the total lipid content of *G. candidum* cells, indicating the destruction of cell membrane structures. (**Zhou *et al.*, 2014**). **Plotto *et al.*,** (**2003**) proved that *T. vulgaris* and *C. citratus* oils, and their respective major components showed complete growth inhibition of *Botrytis cinerea* and *Alternaria arborescens*. Moreover, these authors highlight that *G. candidum* was more sensitive to citral (100% inhibition percentage) and citral-containing oil (100% inhibition percentage) than to *T. vulgaris* oil (81% inhibition percentage) by fumigation. This study showed the capacity of *C. citratus* oil to inhibit conidia germination of isolated pathogens. Similarly, **Jiménez-reyes, (2019)** reported that *Cymbopogon citratus* at 500 ppm, completely inhibits sporulation and germinal tube generation of *Colletotrichum coccodes*, *Botrytis cinerea, Rhizopus stolonifer* and *Cladosporium herbarum* which can cause diseases in tomato fields. To inhibit conidia germination, oil could create lesions on the wall and cell membrane of arthroconidia. This reduce elasticity of membrane thus, increase it rigidity. Moreover, once pass through the cytoplasm, citral might induced interaction with protein-like macromolecules resulting in loss of their biological structure and function (**Luo *et al.*, 2004**).

Cymbopogon citratus oil could be considered as a potential source of sustainable fungicide against *G. candidum* G1, G2 and G3 associated to *Tuta absoluta* infestation.

CONCLUSION AND PERSPECTIVES

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CONCLUSION

This work aimed to determine the insecticidal and antifungal activities of *Thymus vulgaris* and *Cymbopogon citratus* essential oils against *Tuta absoluta* and *Geotrichum candidum* associated to tomato rot in Foumbot production basin. Accordingly, the following conclusions were drawn:

- ✓ *Tuta absoluta* occurred in all the surveyed fields in different villages with mean incidence and severity of 93.20% and 4.40, respectively;
- ✓ *T. vulgaris* and *C. citratus* essential oils exhibited similar knockdown and insecticidal efficiencies through direct contact and fumigation routes. The resultant biological parameters for *C. citratus* and *T. vulgaris* oils were KD_{50} values of 0.19 µL/mL and 0.59 µL/mL and LD_{50} values of 0.32 µL/mL and 0.61 µL/mL respectively for contact toxicity and KD_{50} of 1.29µL/mL and 2.57 µL/mL and LD_{50} of 1.48 µL/mL and 3.05 µL/mL for *C. citratus* and *T. vulgaris* oils respectively for fumigant toxicity;
- ✓ Both essential oils inhibited the mycelia growth of *G. candidum G1, G2 and G3* associated to *Tuta absoluta* infestation with MICs of 0.71 µL/mL and 5.67 µL/mL for *C. citratus* and *T. vulgaris* oils respectively. Moreover, *C. citratus* oil inhibited conidia germination of pathogens with MICs vary from 0.71 µL/mL to 5.67 µL/mL while the MIC of *T. vulgaris* oil was undetermined.

The results obtained revealed that *C. citratus* oil could be employed in the control of *Tuta absoluta* and *Geotrichum candidum*.

PERSPECTIVES

The obtained results clearly show the need to carry out further investigations. Hence, we intend to:

- Survey on *T. absoluta* in other tomato production hotspots in Cameroon;
- Determine the insecticidal and antifungal potentials of *C. citratus* oil against *T. absoluta* and *G. candidum* respectively under greenhouse conditions;
- Undergo formulation of *C. citratus* EO-based bio-insecticide and bio-fungicide for efficient control of *T. absoluta* and *G. candidum*.

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APPENDICES

APPENDICES

Appendice 1: Questionnaire on farmer's perception of *T. absoluta* in Foumbot tomato production basin

LABORATOIRE DE PHYTOBIOCHIMIE ET D'ETUDE DES PLANTES MEDICINALES

LABORATORY FOR PHYTOBIOCHEMISTRY AND MEDICINAL PLANTS STUDIES

QUESTIONNAIRE AUX EXPLOITANTS AGRICOLES

Evaluation de la perception de *Tuta absoluta* par les cultivateurs de tomate du bassin de production de Foumbot

Objectif principal

Suites aux dômmages constatés dans les bassins de production de la tomate au Cameroun parmi lesquels Foumbot, la mineuse de la tomate a été incriminée. Ainsi, la présente enquête a été initiée dans l'objectif d'évaluer les dégâts causés par ce ravageur et de mettre au point une stratégie de lutte efficace.

Strictement confidentiel

La fourniture des informations se fait avec le consentement de l'enquêté et en toute liberté puis les données recueillies sont strictement confidentielles.

I-INFORMATIONS GENERALES

1. Date :…… /…….. /……..
2. Village……..
3. Coordonnées GPS (latitude et longitude)……
4. Altitude du site (en mAsl)……..

II-Caracteristiques du cultivateur

1. **Sexe**

   a.Masculin ☐ b. Féminin ☐

2. **Age**

   a. De 20 à 32 ans ☐ b. De 33 à 45 ans ☐

   c. Plus de 45 ans ☐

3. **Téléphone**……

4. **Nombre de personnes à charge**.....

5. **Niveau d'instruction** :

a. Néant ☐ b. Primaire ☐

c. Secondaire ☐ d. Universitaire ☐

6. **Spécialisation**

a. Agriculture ☐ b. Autres (spécifier)…….

7. **Activité principale**

a. Agriculture ☐ b. Commerce ☐ c. Elevage ☐

Autre……

8. **Cultivez-vous uniquement la tomate ?**

a. Oui ☐ b. Non ☐

Si non, quel autre culture faites-vous ?.....

9. **Depuis combien de temps cultivez-vous la tomate ?** .....

10. **Pour quelle raison cultivez-vous la tomate ?**

a. Commerce ☐ b. Consommation ☐

3.Autre……

11. **Quel rang occupe la tomate dans vos revenus agricoles?**

a. 1er ☐ b. 2ème ☐ c.3ème ☐ d.Autre……

12. **La culture se fait de quel mois à quel autre mois et pourqoui?**
……

13. **Quelle est la superficie cultivée** ?.....

14. **Quelle variété cultivez-vous** ?……

15. **Pourqoui cette variété et pas une autre ?**.....

16. **Combien de fois visitez-vous vos champs ?**.....

17. Faites-vous appel aux spécialistes ou techniciens d'agriculture ?

a. Oui ☐ b. Non ☐

18. **Etes-vous membre d'un regroupement de producteur**

a. Oui ☐ b. Non ☐

Si oui, Lequel ?.....

Quel est le but de votre Association ?.....

Si non Pourquoi ?.....

19. **Quelle a été votre production dans la superficie cultivée ?** :

a. En 2016/2017……..

b. En 2017/2018……..

c. En 2018/2019 …….

III. Perception de Boko-Haram et mesures de contrôle appliquées

1. Quelles sont les principales menaces liées à la culture de la tomate ?

a. Insectes ☐ b. Maladie ☐ c. Autre……

2. Connaissez-vous « Boko-Haram » ?

a. Oui ☐ b. Non ☐

3. **Donnez la date ou année d'apparition dans le champ** .....

4. **Comment est - il? (description : forme, couleur, etc)** ?.....

5. A quel stade de développement de la culture attaque-il ?

a. Germination ☐ b.végétatif ☐ c. floraison ☐

d. fructification (fruits immature) ☐ e. Stade fruits matures ☐

6. Quelles sont les parties de la plante affectées ?

☐

aTige ☐ b. Feuille ☐ c.Fleur

d.Fruit ☐

7. **Comment se manifeste l'attaque de « Boko-Haram » sur ces parties** ?

a. Décoloration de la feuille (zone blanche, devenant brune, presentant une apparence de brûlée et pouvant contenir ou pas les chenilles) ☐

b. Enroulement des feuilles ☐

c. Trous sur le fuit presentant les symptômes de la pourriture et contenant quelques fois chenilles ☐

d. Feces de couleur noire visible sur les organes de la plante, ☐

e. Croissance chétive de la plante. ☐

8. **A combien chiffrez-vous les pertes liées aux attaques de Boko-Haram »** ?...

1. 10-30% ☐ 3. 52-72% ☐

2. 31-51% ☐ 4. 73-100% ☐

9. A part « Boko-Haram », y a-t-il autre(s) insecte(s) qui menace(nt) la culture de la tomate ?

a. Oui ☐ ☐ b. Non

Si oui, Pouvez-vous les citez ?.....

10. Comment luttez-vous contre « Boko-Haram » ?

a. Methode traditionnelle (application du sel, de la cendre, pulverisation d urine de vâche) ☐

b. Enlèvement et brûlure des organes des plants infestés ☐

c. Rotation des cultures ☐

d. Utilisation des produits chimiques ☐

e. Autres…….

11. **Quel(s) insecticide(s) utilisez-vous** ?.....

12. **A quelle dose appliquez-vous cet (ces) insecticide(s)** ?.....

13. **Combien de fois appliquez-vous cet (ces) insecticide(s) ?**.....

14. **A quel moment décidez-vous de traiter votre parcelle contre cet (ces) insecticide(s)** ?

a. Avant les symptômes ☐ b. Après les symptômes ☐

……..

15. **Ce moyen de lutte vous coûtent combien** ?.....

16. Est-il efficace ?

a. Oui ☐ b. Non ☐

17. **Sinon, comment pensez-vous protéger votre culture à long terme** ?.....

Signature et nom de l' informateur

Appendice 2: Clevenger type apparatus

Appendice 3: Composition of culture media

Patato Dextrose Agar (PDA)

Potato extract…….4.0g

Dextrose……..20.0g

Agar…….15.0g

Distilled water……..1 liter

Final pH…… 5.6±0.2 (25 °C)

Preparation : Solve 39 g of lyophilized medium in 1 liter of distilled water, homogenize then supplemented with 0.5 g of Chloramphenicol to inhibit any bacterial growth and bring to the boil until completely dissolved. Distribute in volumes in the appropriate bottles. Sterilize for 15 min at 121 ° C in an autoclave.

Patato Dextrose (PD)

Potato extract…….4.0g

Dextrose……..20.0g

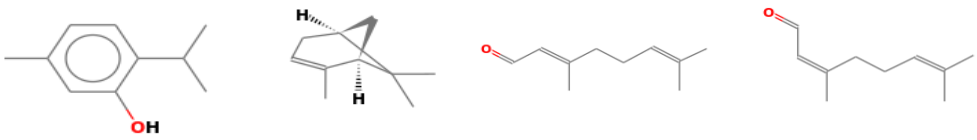
Distilled water……..1 liter

Final pH……5.6±0.2 (25 °C)

Preparation : Solve 24.0 g in 1 litre distilled water, homogenize then supplemented with 0.5 g of Chloramphenicol. Autoclave 15 minutes at 121°C.

Preparation of physiologic liquid : Dissolve 9g of sodium chloride into 1 liter of distilled water then, homogenize and sterilize in an autoclave for 15 min at 121 ° C. Thereafter the solution was cooled in a sterile environment.

Appendice 4: Chemical structures of majors compounds of essential oils



Thymol α-Pinene (dextro) Geranial Neral

Appendice 5: larval mortality as a function of *C. citratus* essential oil doses by contact after four hours of exposure

0.4µL/mL **0.8µL/mL** **1.6µL/mL** **3.2µL/mL** **6.4µL/mL** **Tween 80(0.9%)** **Lynx(3.33mL/L)**

Appendice 6: larval knockdown (%) as a function of *C. citratus* and *T. vulgaris* essential oils doses by contact after four hours of exposure

Products	**C. citratus**	**T. vulgaris**	**Tween(80)**	**Lynx**
Concentrations	**0.4µL/mL**	**0.4µL/mL**	**0.9%**	**3.33mL/L**
	67.50±9.57	22.50±5.00	0.00±0.00	0.00±0.00
Concentrations	**0.8µL/mL**	**0.8µL/mL**	**0.9%**	**3.33mL/L**
	70.00±29.44	77.50±20.62	0.00±0.00	0.00±0.00
Concentrations	**1.6µL/mL**	**1.6µL/mL**	**0.9%**	**3.33mL/L**
	95.00±5.77	87.50±25.00	0.00±0.00	0.00±0.00

Appendice 7: larval knockdown (%) as a function of *C. citratus* and *T. vulgaris* essential oils doses by fumigant toxicity after four hours of exposure

Products	**C. citratus**	**T. vulgaris**	**Tween(80)**	**Lynx**
Concentrations	**0.4µL/mL**	**0.4µL/mL**	**0.9%**	**3.33mL/L**
	6.25±2.00	0.00±0.00	0.00±0.00	0.00±0.00
Concentrations	**0.8µL/ML**	**0.8µL/mL**	**0.9%**	**3.33mL/L**
	12.50±4.00	0.00±0.00	0.00±0.00	0.00±0.00
Concentrations	**1.6µL/ML**	**1.6µL/mL**	**0.9%**	**3.33mL/L**
	62.50±15.00	2.50±1.00	0.00±0.00	0.00±0.00
Concentrations	**3.2µL/mL**	**3.2µL/mL**	**0.9%**	**3.33mL/L**
	100.00±0.00	77.50±28.72	0.00±0.00	0.00±0.00

Appendice 8: *Tuta absoluta* larval duration (days) through essential oils by contact toxicity

Products	**C. citratus oil**	**T. vulgaris oil**	**Tween(80)**	**Lynx**
Concentrations	**0.4µL/mL**	**0.4µL/mL**	**0.9%**	**3.33mL/L**
	2.545±0.688	3.000±0.866	2.742±0.445	2.649±0.753
Concentrations	**0.8µL/mL**	**0.8µL/Ml**	**0.9%**	**3333.33µL/L**
	3.000±0.688	3.667±0.866	2.742±0.445	2.649±0.753

Appendice 9: *Tuta absoluta* pupal duration (days) through essential oils by contact toxicity

Products	**C. citratus oil**	**T. vulgaris oil**	**Tween(80)**	**Lynx**
Concentrations	**0.4µL/mL**	**0.4µL/mL**	**0.9%**	**3.33mL/L**
	7.00±0.89	7.22±0.67	6.48±0.81	6.70±0.52
Concentrations	**0.8µL/mL**	**0.8µL/mL**	**0.9%**	**3.33mL/L**
	8.00±0.00	6.67±1.16	6.48±0.81	6.70±0.52

Appendice 10: *Tuta absoluta* larval duration (days) through essential oils by fumigant toxicity

Products	**C. citratus oil**	**T. vulgaris oil**	**Tween(80)**	**Lynx**
Concentrations	**0.4µL/mL**	**0.4µL/mL**	**0.9%**	**3.33mL/L**
	1.25±0.25	1.21±0.25	2.36±0.78	2.26±0.44
Concentrations	**0.8µL/mL**	**0.8µL/mL**	**0.9%**	**3.33mL/L**
	2.58±0.50	2.43±0.50	2.36±0.78	2.26±0.44
Concentrations	**1.6µL/mL**	**1.6µL/ML**	**0.9%**	**3.33mL/L**
	3.00±0.00	3.29±0.72	2.36±0.78	2.26±0.44

Appendice 9: *Tuta absoluta* pupal duration (days) through essential oils by fumigant toxicity

Products	**C. citratus oil**	**T. vulgaris oil**	**Tween(80)**	**Lynx**
Concentrations	**0.4µL/mL**	**0.4µL/mL**	**0.9%**	**3.33mL/L**
	4.46±0.10	3.83±0.27	7.37±0.94	7.00±0.65
Concentrations	**0.8µL/mL**	**0.8µL/mL**	**0.9%**	**3.33mL/L**
	8.96±0.21	7.73±0.53	7.37±0.94	7.00±0.65
Concentrations	**1.6µL/mL**	**1.6µL/mL**	**0.9%**	**3.33mL/L**
	9.00±0.00	7.89±1.02	2.36±0.78	2.26±0.44

Appendice 10: Mycelia growth inhibition percentage of *C. citratus* oil against *G. candidum G1, G2 and G3*

Concentrations (µL/mL)	***G. candidum* G1**	***G. candidum* G2**	***G. candidum* G3**
0.09	16.23±4.94	6.36±2.11	4.83±2.74
0.18	53.07±3.74	41.29±3.04	20.61±2.01
0.35	64.29±0.00	63.49±2.75	47.22±7.56
0.71	100±0	100±0	100±0
1.42	100±0	100±0	100±0

Appendice 11: Mycelia growth inhibition percentage of *T. vulgaris* oil against *G. candidum G1, G2 and G3*

Concentrations (µL /mL)	***G. candidum* G1**	***G. candidum* G2**	***G. candidum* G3**
0.35	30.14±0.93	49.80±6.79	71.59±1.97
0.71	87.40±0.17	56.47±0.00	90.91±1.74
1.42	94.27±0.08	88.63±0.68	93.18±1.97
2.83	94.66±1.29	94.12±2.04	95.45±0.00
5.67	100±0	100±0	100±0

Appendice 12: Spore germination inhibition percentage of *C. citratus* oil against *G. candidum G1, G2 and G3*

Concentrations (µL /mL)	***G. candidum* G1**	***G. candidum* G2**	***G. candidum* G3**
0.09	65.09±0.000	62.69±1.75	80±0
0.18	67.76±7.56	85.63±5.35	92.35±0.00
0.35	80.51±1.46	88.50±1.74	99.33±1.74
0.71	92.02±0.00	99.81±0.00	100±0
1.42	91.92±0.00	100±0	100±0
2.83	93.85±0.00	100±0	100±0
5.67	100±0	100±0	100±0

Appendice 13: Spore germination inhibition percentage of *T. vulgaris* oil against *G. candidum G1, G2 and G3*

Concentrations (µL /mL)	***G. candidum* G1**	***G. candidum* G2**	***G. candidum* G3**
0.09	50.10±1.40	58.64±0.00	46±1.73
0.18	55.29±0.00	65.13±6.39	66.75±1.65
0.35	69.72±0.18	91.36±0.41	67.00±2.03
0.71	94.99±3.11	91.97±0.13	70.72±0.32
1.42	94.07±0.08	93.03±0.00	78.39±2.61
2.83	94.97±0.00	93.19±1.74	87.29±2.46
5.67	90.09±0.00	92.81±0.07	87.79±0.00
11.33	93.27±0.00	92.78±0.07	89.88±0.84