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EVALUATION OF THREE NATURAL ENEMIES: PHYTOSEIULUS PERSIMILIS, AMBLYSEIUS SWIRSKII, AND BEAUVERIA PSEUDOBASSIANA, FOR THE INTEGRATED MANAGEMENT OF GREENHOUSE VEGETABLE ARTHROPOD PESTS

By

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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Agricultural Sciences
of the Faculty of Agricultural and Food Sciences
at the American University of Beirut

Beirut, Lebanon
January, 2018

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ACKNOWLEDGMENTS

I owe my family members the honor of successfully completing my master's degree. I wouldn't have accomplished my goal without their ongoing love, support, and encouragement.

I sincerely thank my advisor Dr. Youssef Abou Jawdeh for guiding me through my dissertation and helping me overcome several obstacles I encountered during my research. I would like to present him with my gratitude and appreciation for being a successful and compassionate academic advisor.

I would also like to thank my co-advisors Dr. Nabil Nemer, Dr. Isam Bashour, and Dr. Khouzama Knio for further assisting me during my research.

Special thanks to Dr. Houssam Shaib and Dr. Samer Kharroubi for helping me conduct statistical analysis of the data presented in my research.

My appreciation goes to the lab members Miss Hana Sobh, Vicken Aknadibossian, Mira Yehya, Fatima Abdallah, Nour Ezzedine and to all my friends at AUB for making this 2-year experience easy, enjoyable and informative.

Finally, I dedicate my master's thesis to Patil Tawidian. Your support, care, and love allowed me to reach this point in my academic career. Thank you for being in my life.

AN ABSTRACT OF THE THESIS OF

Andre Elias Abou Haidar for Master of Science
Major: Plant Protection

Title: Evaluation of three natural enemies: *Phytoseiulus persimilis*, *Amblyseius swirskii*, and *Beauveria pseudobassiana*, for the integrated management of greenhouse vegetable arthropod pests

Biologically-based integrated pest management (IPM) has become one of the main pest control strategies on high-value agricultural crops. However, in Lebanon, most of the farmers rely on frequent application of pesticides due to lack of alternative and effective pest control approaches. For that reason, field studies were conducted to assess the control efficacy of *Amblyseius swirskii* and *Phytoseiulus persimilis* for the management of greenhouse cucumber arthropod pests. The laboratory study focused on the use of *Beauveria pseudobassiana* for the control of *Tuta absoluta*. To test the efficacy of the natural enemies, two greenhouses were selected at each of the two selected experimental sites. One greenhouse served as a control where the farmer followed his normal plant protection practices while in the other, IPM practices were implemented including natural enemy releases. The populations of insects and mites were recorded on cucumber leaves and/or flowers. In the IPM greenhouses, *A. swirskii* successfully maintained whitefly nymph and thrips populations below their economic threshold levels, during most of the growing period. Moreover, *P. persimilis*, applied at the proper time, was highly effective in reducing spider-mite infestations. In the control greenhouse, at site A (Tamich), whitefly populations were maintained at a very low level while thrips and mite populations exceeded the ETLs, despite of 8 insecticidal/acaricidal sprays. On the other hand, at site B (Zahrani), the farmer was able to supress all arthropod pest populations below the ETLs due to heavy pesticide applications, which totaled 15 sprays each consisting of mixtures of a minimum of three pesticides. To test the efficacy of *B. pseudobassiana*, different conidial suspensions, ammended with or without adjuvants, were sprayed on *T. absoluta* eggs placed on detached tomato leaves in Petri-dishes. The results showed that the entomopathogenic fungus was highly effective in controlling the egg and larval stages of *T. absoluta*, in a dose-dependant infection model. The control activity of the local fungal isolate was improved by the addition of 1% corn oil to the conidial suspensions. The highest corrected mortality (CM), of more than 92%, was recorded on eggs treated with the highest conidial suspension (10^8 conidia mL-1) + 0.01% Tween 20 + 1% corn oil. This research proved the high potential for the use of predators and parasites to control arthropod pest populations, with a considerable reduction in insecticidal/acaricidal sprays ranging from 63% to 93%.

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ABBREVIATIONS

%	Percent of a hundred
β	Beta
°C	Degree Celsius
?	Unkown toxicity
m^2	Square meter
cm	Centimeter(s)
mm	Millimeter(s)
µm	Micrometer(s)
L	Liter(s)
mL	Milliliter(s)
Kg	Kilogram(s)
g	Gram(s)
mg	Milligram(s)
A. colemani	*Aphidius colemani*
A. gossypii	*Aphis gossypii*
a.i.	Active ingredient
ANOVA	Analysis Of Variance
A. swirskii	*Amblyseius swirskii*
B. bassiana	*Beauveria bassiana*
B. pseudobassiana	*Beauveria pseudobassiana*

BSC	Blue sticky cards
B. tabaci	*Bemisia tabaci*
B. thuringiensis	*Bacillus thuringiensis*
CABYV	*Cucurbit aphid-borne yellows virus*
CCYV	*Cucurbit chlorotic yellows virus*
CM	Corrected mortality
CMV	*Cucumber mosaic virus*
C. tannourinensis	*Cephalcia tannourinensis*
CVYV	*Cucumber vein yello virus*
CYSDV	*Cucurbit yellow stunting disorder virus*
DD	Degree days
E. eremicus	*Eretmocerus eremicus*
EIL	Economic injury level
et al.	*et alia* (and others)
ETL	Economic threshold level
EW	Emulsion, oil in water
FAO	Food and Agriculture Organization
F. occidentalis	*Franklinella occidentalis*
F. oxysporum	*Fusarium oxysporum*
Ha	Hectare
Hrs	Hours
IGRs	Insect growth regulators
IPM	Integrated Pest Management

ITS	Internal Transcribed Spacer
L:D	Light to Dark ratio
L. huidobrensis	*Liriomyza huidobrensis*
LMV	*Lettuce mosaic virus*
Log	Logarithm
LSD	Least significant difference
M	Million
MD	Moderately toxic
MEAM 1	Middle East-Asia Minor 1
MED	Medditteranean
M. persicae	*Myzus persicae*
M. pygmaeus	*Macrolophus pygmaeus*
NT	Not toxic
P. aphanidermatum	*Pythium aphanidermatum*
P. cubensis	*Pseudoperonospora cubensis*
PDA	Potato Dextrose Agar
P. fusca	*Podosphaera fusca*
PM	Potted marigolds
P. persimilis	*Phytoseiulus persimilis*
PRSV	*Papaya ringspot virus*
PVY	*Potato virus Y*
®	Registered trade mark
SC	Suspension concentrate

SDA	Sabouraud's Dextrose Agar
S. muricatum	*Solanum muricatum*
Spp.	Species
ST	Slightly toxic
S. tuberosum	*Solanum tuberosum*
Subsp.	Subspecies
T	Toxic
T. absoluta	*Tuta absoluta*
TSWV	*Tomato spotted wilt virus*
T. urticae	*Tetranychus urticae*
T. vaporariorum	*Trialeurodes vaporariorum*
V. dahliae	*Verticillium dahliae*
WMV	*Watermelon mosaic virus*
WP	Wettable powder
YSCs:	Yellow sticky cards
ZYMV	*Zucchini yellow mosaic virus*

CHAPTER I

INTRODUCTION

In Lebanon, tomato (*Solanum lycopersicum*, Linnaeus) and cucumber (*Cucumis sativus,* Linn) are the most commonly produced vegetable crops. Several biotic and abiotic factors may adversly affect the yield potential of vegetables; including temperature and moisture stress, nutrient deficiencies, disease infections, weed and insect/mite infestations (Rubatzky & Yamaguchi, 2012). Pest attack contribute to significant yield losses, which could reach up to 70%, if not effectively managed. On average, potential yield loss attributted to pest damage is estimated around 35% (Popp et al., 2013).

Out of the several known insect pests on vegetables, whiteflies (*Trialeurodes vaporariorum* Westwood and *Bemisia tabaci* Gennadius), two-spotted red mites (*Tetranychus urticae* Koch), thrips (*Thrips tabaci* Lindeman and *Frankliniella occidentalis* Pergande), and aphids (*Aphis gossypi* Glover and *Myzus persicae* Sulzer) are considered the major and most devastating arthropod pests on cucumber plants; which could incur dramatic yield losses (Shipp et al., 1991). In addition to the previously listed pests, *Tuta absoluta* Meyrick is reported as a destructive gelechiid moth on tomatoes. The main pest management strategy adopted by farmers, to reduce insect populations to below economic threshold levels, is most often confined to the application of insecticides (Desneux et al., 2010). However, the over reliance on synthetic chemicals, often without alternation of pesticides with different modes of action, has led to development of insect resistance against most of the insecticides commonly applied (Desneux et al., 2010; Guedes &

Picanco, 2012). Therefore, alternative management techniques should be implemented for satisfactory pest control. IPM has been recognized as an effective strategy to supress pest population, mainly through the integration of several environmently friendly control techniques (Parsa et al., 2014), while minimizing the potential risk of development of insect/mite resistance by reducing the number of chemical sprays and alternating between pesticides with different modes of action (Barzman et al., 2015).

This study aims at:

1. Evaluating under normal greenhouse production conditions the efficacy of natural enemies, namely *Amblyseius swirskii*, *Eretmocerus eremicus*, and *Phytoseiulus persimilis* for the management of cucumber greenhouse pests
2. Assessing under laboratory conditions the efficacy of a Lebanese fungal isolate; *Beauveria pseudobassiana*, against the lepidopterous tomato leafminer; *Tuta absoluta.*

CHAPTER II

LITERATURE REVIEW

A. Overview of Protected Cultivation

Primitive greenhouse production techniques dated back to late 15^{th} to 18^{th} centuries in Europe and China. The rise of commercial greenhouse production in Northern Europe started in the early 20^{th} century (Pardossi et al., 2004; Wittwer & Castilla, 1995). In mid-20^{th} century, protected cultivation was introduced to the Mediterranean regions reaching a total area of 200,000 Ha in 1997 (De Pascale & Maggio, 2004). A total protected cultivation area of 33,990 Ha was recorded in Egypt, Jordan, Kuwait, Lebanon, Libya, Morocco, Qatar, Syria, Tunisia, Turkey, United Arab Emirates and Yemen during years 2007-2008 (Baudoin et al., 2013).

Greenhouse production offers superior quality vegetables, efficient use of agricultural resources namely labor, water, fertilizers, and pesticides along with protection against harsh environmental conditions. Thus, enabling farmers to grow crops all year round (Cantliffe et al., 2001; Pardossi et al., 2004). Moreover, greenhouses fitted with properly maintained insect-proof nets can decrease pest infestation, which in turn minimize the use of insecticides (Cantliffe et al., 2001).

The major crops produced under protected cultivation are those belonging to the Solanaceae and Cucurbitaceae families mainly; pepper, tomato, eggplant, cucumber, watermelon, melon, and zucchini along with cut flowers, strawberries, ornamentals, and few fruit crops (Albajes et al., 2000; Gerson & Weintraub, 2007; Pardossi et al., 2004).

B. Cucumber production in Lebanon

Cucurbits refer to all plant species belonging to the family Cucurbitaceae which encompasses around 110 to 130 genera with 650 to 850 species (Rahman et al., 2006; Weng & Sun, 2011). The main crops cultivated within the latter family are cucumbers (*Cucumis sativus* L.*)*, melons (*Cucumis melo* L.*)*, watermelons *(Citrulus lanatus* Thunb*)* and pumpkins *(Cucurbita maxima* Duch*)* (Weng & Sun, 2011). Cucurbits have high nutritional value and are rich in β-carotene, Vitamin A and potassium (Abrahamian, 2013). Cucumber; the most commonly grown greenhouse vegetable in the world, is an annual, cold-sensitive crop that grows best at temperatures ranging between 24°C and 29°C (Jeffrey, 1980; Mohammadi & Omid, 2010). During 2016, world cucumber and gherkins production reached around 80.6 M tons with a cultivated area of 2.14 M Ha, with China being the lead producing country. In Lebanon, total cucumber and gherkins production reached 127,850 tonnes on a cultivated area of 3,045 Ha (FAOSTAT, 2016).

1. *Cucumber insect/mite pests in Lebanon*

a. Whiteflies

The family Aleyrodidae includes around 120 genera and 1,300 to 1,500 species of 1-3 mm long insects known as whiteflies (Jones, 2003; Navas-Castillo et al., 2011). *T. vaporariorum* and *B. tabaci* (Homoptera: Aleyrodidae) are known to be the most common pests within the latter family with six developmental stages; egg, four larval instars and adult whitefly. At 25°C, the average development time of whiteflies form egg to adult stage is around 20 days on cucumber plants (Malais & Ravensberg, 2004).

The wide host range of the sweet potato whitefly, *B. tabaci*, (more than 700 different plant species) and its high fecundity rate enabled it to be a major pest on agricultural crops in the Middle-East, North and Central America, Europe and the Caribbean basin (Salas & Mendoza, 1995). Out of the several known *B. tabaci* strains, the most common in the Mediterranean regions are Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species (De Barro et al., 2011). Whitefly damage on crops occurs through induction of physiological disorders like 'silver leaf' symptoms on cucurbits by *B. tabaci*, development of sooty mould (Cladosporium spp.) in response to honeydew production, and transmission of more than 100 viral plant diseases (Baroudy, 2010; Jones, 2003; Zhao et al., 2014).

The primary management strategy adopted by farmers to control whiteflies is often limited to the use of chemical insecticides. For instance, bifenthrin, fenpropathrin, endosulfan, acephate, and methamidophos were the most successful pesticides against whiteflies (Palumbo et al., 2001). However, in many vegetable cropping systems, intensive use of insecticides was required to achieve an effective control of *B. tabaci*, which lead to the development of insect resistance to organophosphates, pyrethroids, carbamates and chlorinated hydrocarbons (Elbert & Nauen, 2000; Nauen & Denholm, 2005). After that serious resistance issue, insecticides belonging to insect growth regulators (IGRs) and neonicotinoids, were the most effective against *B. tabaci* (Palumbo et al., 2001). Neonicotinoids, like imidacloprid, affect the insect nervous system by acting on the acetylcholine receptors (Van der Sluijs et al., 2013) whereas, IGRs like buprofezin and pyriproxyfen mimic the action of hormones targeting insect growth and development thus, leading to insect pest suppression (Beckage, 2000). During 1996, the first neonicotinoids resistance was recorded against imidacloprid on *B. tabaci* (Elbert & Nauen, 2000). After which, biotypes MEAM1 and MED developed resistance to several chemicals in the latter class such as thiamethoxam (Bass et al., 2015; Kliot et al., 2016). Resistance against buprofezin was also documented in Europe on *T. vaporariorum* (Gorman et al., 2002). Therefore, an alternative control technique should be adopted to keep *B. tabaci* and *T. vaporariorum* below ETLs (4.6 and 5 whitefly adults per leaf, respectively) (Jeon et al., 2009; Shen et al., 2005).

b. <u>Thrips</u>

Thrips are small insect pests ranging from 0.5 to 5 mm in size. Nine families and around 5,000 species have been recognized, out of which 95% belong to the family Thripidae (Jones, 2005). Until 1980s, onion thrips; *Thrips tabaci* (Thysanoptera: Thripidae), was known as the major glasshouse insect pest. Then the western flower thrips; *Frankliniella occidentalis* (Thysanoptera: Thripidae), originating from America, became the most devastating thrips species worldwide (Albajes et al., 2000; Malais & Ravensberg, 2004) with an ETL of 3 to 7.5 adults per cucumber flower and 20 to 50 adults per sticky trap per day (Shipp et al., 2000) and an EIL of 1.7 thrips adult or 9.5 larvae per cucumber leaf (Jarošík et al., 1997).

The life cycle of western flower thrips is composed of an egg stage, two larval stages, pre-pupa stage, pupal stage and an adult stage with optimum development occurring at 30^{o}C, but a decline is observed at temperatures above 35^{o}C and below 10^{o}C (Malais &

Ravensberg, 2004). Damage caused by thrips can be described as direct through feeding on flowers, fruits, and leaves leading to silver scarring and malformations (Shipp et al., 2000) and indirect through transmitting viral diseases for example; *Tomato spotted wilt virus* (TSWV) on tomatoes (Abou-Jawdah et al., 2006; Jones, 2005).

Over many years, chemical control of thrips has relied on sprays of organophosphates, pyrethroids, carbamates, spinosyns and avermectins (Kliot et al., 2016). Some farmers reported up to 19 sprays at 3-4 days interval for western flower thrips management. Such practices aided a relatively rapid build-up of resistance in thrips populations to the above mentioned insecticides. For instance, resistance of western flower thrips (*F. occidentalis)* to the pesticide toxaphene on cotton was first observed during 1961 (Jensen, 2000).

c. <u>Two-spotted spider mites</u>

Out of the known spider mite families feeding on higher plants, Tetranychidae, Tarsonemidae, and Eriophyoidae pose the greatest threat to the agricultural crops (Jeppson et al., 1975; Malais & Ravensberg, 2004). *T. urticae* (Acari: Tetranychidae) is considered as a destructive greenhouse arthropod pest due to its high fecundity rate and polyphagous feeding habit (Albajes et al., 2000; Malais & Ravensberg, 2004; Sabelis, 1982; Zhang, 2003). Mites belonging to Tetranychidae family have five developmental life stages; egg, larval stage, two nymphal stages and adult stage (Jeppson et al., 1975; Malais & Ravensberg, 2004). *T. urticae* development is optimal at temperatures ranging between 25-30^{o}C along with low relative humidity (RH). Under such conditions, the development time of spider mites from egg to adult stage is between 6 and 10 days. However, its development stops at temperatures below 12^{o} C and is retarded at temperatures above 40^{o} C (Malais & Ravensberg, 2004; Tanigoshi et al., 1975).

All active developmental stages of the two-spotted spider mites damage the host through feeding on the underside of leaves and sucking its sap. As a result, photosynthetic potential of the host decreases and unfavorable symptoms such as spotting and webbing develops on leaves and fruits, leading to unmarketable products (Malais & Ravensberg, 2004; Reddall et al., 2004).

Chemical resistance to acaricides can develop rapidly following the misuse of a newly introduced acaricide (Khalighi et al., 2014). For instance, resistance of *T. urticae* to abamectin and fenbutatin oxide has been reported from all over the world. Korea, Japan, UK, and Australia have reported mite resistance to the mitochondrial electron transport inhibitors (MET1) group of acaricides like pyridaben, fenazaquin, and fenpyroximate (Gorman et al., 2002). Therefore, resistance management strategies should be adopted and implemented by farmers in order to reduce the risk of developing acaricide resistance (Khalighi et al., 2014).

d. <u>Aphids</u>

Aphids belong to family Aphididae which encompasses several species known to inflict significant damage to a broad range of cultivated crops, including many fruit crops, vegetables, and ornamentals. They are classified as harmful agricultural pests due to their feeding habit (piercing sucking) and massive reproductive potential (Dedryver et al., 2010). The most important greenhouse aphid species are the green peach aphid (*M. persicae* subsp. *persicae)*, tobacco aphid (*M. persicae* subsp. *nicotianae)*, cotton aphid (*A. gossypii)* and potato aphid (*Macrosiphum euphorbiae* Thomas) (Malais & Ravensberg, 2004). Their feeding induce hormonal imbalance, which in turn may cause leaf deformation, growth retardation, and yield reduction. Moreover, aphids produce honeydew on which epiphytic fungal growth may develop causing reduction in leaf photosynthesis (Malais & Ravensberg, 2004). They are also vectors of up to 50% of insect-transmitted viruses like *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Lettuce mosaic virus* (LMV), *Papaya ringspot virus* (PRSV) (Dedryver et al., 2010), *Zucchini yellow mosaic virus* (ZWMV), *Watermelon mosaic virus* (WMV) (Perring et al., 1992), and *Cucurbit aphid-borne yellows virus* (CABYV) (Carmo-Sousa et al., 2016).

Growers relied on a large number of insecticides like organophosphates, carbamates, organochlorines and pyrethroids to reduce aphid populations. Neonicotinoids were also used in order to reduce viral-disease infections transmitted by aphids and to avoid direct damage induced by feeding on plant sap (Foster et al., 2000; Voudouris et al., 2016). However, it is known that aphids, specifically green peach aphid, can easily develop resistance to a broad range of insecticides (Devonshire et al., 1998; Foster et al., 2000). It is reported that aphids evolved high tolerance to organophosphates, pyrethroids, carbamates, and neonicotinoids (Foster et al., 2000; Voudouris et al., 2016).

e. Leaf miners

Among approximately 2,500 species in the family Agromyzidae, *Liriomyza spp.* and *Chromatomyia syngenesiae* Hardy are the most common pests. Eleven species of leaf miners are polyphagous with five belonging to the genus *Liriomyza* (Parrella, 1987). *L.*

huidobrensis Blanchard is considered a major pest on cucurbits, solanaceous and leguminous crops along with many others crops (Weintraub & Horowitz, 1995). Direct damage to the host occurs through larval leaf mining which leads to leaf deformation with premature drop and desiccation and yield reduction. Moreover, infestations at early stages may lead to total mortality of crops (Malais & Ravensberg, 2004).

The control of the leaf miner is commonly achieved by intensive use of insecticides. IGR cyromazine and neurotoxin abamectin, are considered effective chemicals which target the larval stage of leaf miners. However, it has been proven that leaf miners can build up tolerance to insecticides (Wei et al., 2015). For instance, abamectin resistance has been reported in several countries. Therefore, application of insecticide with different modes of action is recommended when necessary (Gao et al., 2017).

f. <u>Nematodes</u>

Nematodes are threatening pests in greenhouse crop production, especially in Lebanon and other Middle-Eastern countries (Haroutunian, 2013). Root-knot nematode (*Meloidogyne* Goeldi spp.) and cyst nematodes (*Heterodera* Schmidt and *Globodera* Skarbilovich spp.) are the most economically important nematodes (Urwin et al., 1997). Second juvenile instars of the root knot nematodes damage crops through causing galls or knots formation on plant roots which in turn lead to plant stunting and yield reduction (Bird, 1974).

Prior to planting, methyl bromide, a broad- spectrum soil fumigant, was applied to control pests including nematodes and soil-borne diseases. It was intensively used by farmers due to its high efficacy in managing the above mentioned agricultural problems;

however due to environmental and health hazards it is officially banned in all countries (Anou-Jawdah et al., 2000; Giannakou et al., 2002; Mouttet et al., 2014). Many other nematicides that were used in the field or in greenhouse were also banned. Therefore, other management techniques should be implemented to reduce such risks (Khurma & Mangotra, 2004; Patel & Dhillon, 2017; Urwin et al., 1997).

Table 1. Summary of major cucumber arthropod pests in Lebanon

Arthropod pests	**Major species**	**Optimal T (°C)**	**Major Characteristics**	**Damage**	**ETL**
Whiteflies	*Bemisia tabaci* & *Trialeurodes vaporariorum*	25-30 °C	Wide host range; high fecundity rate; ability to tolerate high T°C	Induction of physiological disorder; development of sooty mould; transmission of viral diseases	5 Whitefly adults per leaf
Thrips	*Frankliniella occidentalis* & *Thrips tabaci*	25-30 °C	Wide host range; high fecundity rate	Feeding on leaves, flowers, and fruits; transmission of viral diseases	1.2 Thrips per leaf or > 3 Thrips per flower
Spider-mites	*Tetranychus urticae*	25-30 °C	Wide host range; high fecundity rate; ability to tolerate high T	Reduce photosynthetic potential of plants; development of unfavorable symptoms on leaves and fruits	2 Spider-mites per leaf
Aphids	*Myzus persicae* & *Aphis gossypii*	25-30 °C	Short generation time; rapid rate of reproduction	Leaf deformation; growth retardation; development of sooty mould; transmission of viral diseases	Not determined

2. ***Major diseases on cucumbers in Lebanon***

a. Fungal diseases

i. Powdery mildew

Powdery mildew is a fungal disease infecting a broad range of crop families including Cucurbitaceae, Asteracea, Lamiaceae, Solanaceae, Scrophulariaceae, and Verbenaceae (Perez-Garcia et al., 2009).

Podosphaera fusca; also known as *Sphaerotheca fuliginea,* is the main causal agent of powdery mildew disease on cucurbits. At a lesser extent, the disease can also be caused by *Golovinomyces cichoracearum* (Fukino et al., 2013). *P. fusca* is an obligate parasite belonging to Phylum Ascomycota and Family Erysiphaceae. It is easily detected due to visible powdery, white mycelial growth colonizing leaf, stem and petiole surfaces of the host. Under suitable conditions, *P. fusca* completely covers leaf surfaces, reduces photosynthesis, and leads to chlorosis and early drop of diseased leaves. If not effectively treated, cucurbits record dramatic yield losses upon severe fungal infection (Perez-Garcia et al., 2009). Most common practices for the management of powdery mildew are fungicide applications and growing resistant/tolerant varieties (Fukino et al., 2013).

ii. Downy mildew

Downy mildew disease infects around 50 plant species in almost 20 genera within Cucurbitaceae family including cucumbers, pumpkin, watermelon and many others, especially in humid areas (Oerke et al., 2006; Savory et al., 2011). The disease is caused by *Pseudoperonospora cubensis*; an obligate plant parasite belonging to Phylum Oomycota and Family Peronosporaceae. Several factors including leaf wetness, temperature, and inoculum level are essential for fungal infection. For instance, at a temperature of 20°C and in the presence of high inoculum level, *P. cubensis* infection starts after a minimum of 2 hours of leaf wetness. As temperature decreases, the number of hours of leaf wetness required for infection increases (Cohen, 1977). Disease symptoms vary depending on the host specie and variety (Lebeda & Cohen, 2011). On cucumber foliage, angular yellow lesions restricted by leaf veins develop, expand with disease progression and lead to shriveled and dead leaves. Thus with reduction in plant canopy, cucurbit yield and quality significantly decreases (Savory et al., 2011).

For effective management of downy mildew, an integration of fungicide application, cultural practices, and resistant varieties should be implemented since resistance to fungicides like metalaxyl and its active form mefenoxam has been reported in several countries (Savory et al., 2011).

iii. Fusarium wilt

Fusarium wilt on cucumbers, caused by *Fusarium oxysporum* f. sp. *cucumerinum,* is a devastating soil-borne fungal disease, leading to significant yield losses that could reach up to 50% and to total crop failure in certain adopted cultivation systems, like long term monoculture (Yang et al., 2011). *F. oxysporum* belongs to phylum Ascomycota and Family Nectriaceae. It has a wide host range including tomato, cucumber, bean, melon, cotton, and banana, and each host genus is attacked by a different forma specialis (Michielse & Rep, 2009). The causal agent can infect plant vascular system and release mycotoxins into crop tissues, which in turn lead to growth stunting, chlorosis of older leaves, and wilting followed by crop death (Michielse & Rep, 2009; Yang et al., 2011). The most common practices employed to control *F. oxysporum* include soil sterilization and growing resistant plant cultivars (Michielse & Rep, 2009).

iv. Verticillium wilt

Verticillium wilt, belonging to Phylum Deuteromycota and Family Plectosphaerellaceae, is another major soil-borne disease adversely affecting around 400 dicotyledonous plant hosts. Out of the ten identified *Verticillium* species, *V. dahliae* is considered as the major pathogen infecting a wide host range of plants (almost 200 host species in more than 14 plant families) (Inderbitzin et al., 2014). The disease is characterized by the yellowing of older leaves which dry progressively and wilt. Under severe infection, the plant dies within few weeks. Considerable yield reduction, exceeding 50%, has been reported on certain agricultural crops grown on *Verticillium*-contaminated soil like potatoes and may reach up to 100% in lettuce (Klosterman et al., 2009). The ability of *V. dahliae* to overwinter in soils or on plant debris as microsclerotia for over 14 years without the presence of a host is what makes the disease difficult to control (Inderbitzin et al., 2014). Verticillium wilt management was highly dependent on soil fumigation with methyl bromide since crop rotation is an unsuccessful control practice due to the wide host range of the pathogen (Klosterman et al., 2009) and plant resistance to *V. dahlia* is uncommon (Faino et al., 2015). However, quarantine restriction on the movement of plant material is an effective practice to avoid the contamination of the soil by *V. dahliae.* Moreover, studies conducted on the application of organic amendments containing proteins or fatty acids, showed a potential to reduce the disease incidence (Klosterman et al., 2009).

v. Damping-off

Damping off is a fungal disease affecting a wide range of high-value agricultural crops belonging to several families, including Cucurbitaceae and solanaceae (Fukuta et al., 2013). The disease can be caused by several fungi, most importantly by *Pythium aphanidermatum* which belongs to Phylum Oomycota and Family Pythiaceae (Adams, 1971). It is favored by a temperature ranging from 30°C to 35°C and high RH. Under optimal environmental conditions, *P. aphanidermatum* inflicts significant damage to host plants by attacking their roots and root tips leading to water-soaked appearance of infected seedlings which in turn collapse and die (Fukuta et al., 2013). To avoid such obstacle, farmers usually apply fungicides like propamocarb hydrochloride and metalaxyl (Nzungize et al., 2012). However, resistance to chemicals is much likely to develop. Therefore, an IPM strategy such as deep plowing, use of raised beds, and growing tolerant cultivars, should be implemented to avoid the risk of developing disease resistance (Al-Balushi et al., 2018).

Table 2. Summary of major fungal diseases infecting cucumber plants in Lebanon

Fungal disease	**Major causal agent**	**Phylum**	**Family**	**Symptoms & Damage**	**Management**
Powdery mildew	*Podosphaera fusca*	Ascomycota	Erysiphaceae	Powdery, white mycelial growth on leaf, stem and petiole; chlorosis; early drop of diseased leaves; reduction in photosynthesis	Fungicide applications; resistant/tolerant varieties
Downy mildew	*Pseudoperonospora cubensis*	Oomycota	Peronosporaceae	Angular yellow lesions restricted by leaf veins; shriveled and dead leaves; reduction in plant canopy	Fungicide application; cultural practices; resistant varieties
Fusarium wilt	*Fusarium oxysporum* f. sp. *cucumerinum*	Ascomycota	Nectriaceae	Infect plant vascular system; release mycotoxins into crop tissues; growth stunting; chlorosis of older leaves; wilting	Soil solarization; resistant plant cultivars
Verticillium wilt	*Verticillium dahliae*	Deuteromycota	Plectosphaerellaceae	Infect plant vascular system; chlorosis and wilting of older leaves; death of infected plants	Quarantine; organic amendments
Damping off	*Pythium aphanidermatum*	Oomycota	Pythiaceae	Water-soaked appearance of infected seedlings; death of plants	Deep plowing; raised beds; growing tolerant cultivars; fungicides

b. Viral diseases

i. *Cucurbit yellow stunting disorder virus*

Cucurbit yellow stunting disorder disease is caused by *Cucurbit yellow stunting disorder virus* (CYSDV; genus *Crinivirus*, family: *Closteroviridae*). CYSDV is transmitted by the whitefly *B. tabaci* biotypes MEAM1 and MED, in a semi-persistent manner. During 1990s, CYSDV was reported in the Mediterranean region, including Lebanon (Eid et al., 2006). Visible disease symptoms develop four weeks after initial infection and are characterized by interveinal chlorosis, thickness and brittleness of older leaves, and plant growth stunting. As a result of viral infection, significant yield reduction, ranging from 30% to 50%, takes place (Eid et al., 2006; Louro et al., 2000). Management of CYSDV is restricted to resistant/tolerant plant varieties, control of viral vector, and preventive cultural practices like installing insect-proof nets or silver mulch to repel whiteflies (Adkins et al., 2011; Eid et al., 2006).

ii. *Zucchini yellow mosaic virus*

Zucchini yellow mosaic virus (ZYMV; genus *Potyvirus*, family *Potyviridae)* is a destructive pathogen of crops in the Cucurbitaceae family. It is transmitted mainly by *M. persicae* and *A. gossypii,* in a non-circulative manner. The virus is also transmitted by infected seeds. ZYMV is distributed all over the world (Desbiez & Lecoq, 1997) and was first reported in Lebanon during 1979 on cucumber crops (Lesemann et al., 1983). The disease is characterized by chlorotic mottling, deformation of leaves and fruits, and plant stunting (Desbiez & Lecoq, 1997; Simmons et al., 2013). Control measures for ZYMV include cultural practices like silver plastic mulch and floating row covers to reduce viral transmission by aphids and cross-protection with a mild strain (Abou-Jawdah et al., 2000).

iii. *Cucurbit chlorotic yellows virus*

Cucurbit chlorotic yellows virus (CCYV; genus *Crinivirus*, *family Closteroviridae*) is an economically important virus having a wide host range. It is transmitted, in a semi-persistent manner, by both biotypes MEAM1 and MED of *B. tabaci.* After its first identification in Japan, in 2004 (Kubota et al., 2011), it was reported in Lebanon in 2011 on cucumber plants (Abrahamian et al., 2012). The symptoms of CCYV start as yellow leaf spots that develop into total yellowing of lower leaves (Kubota et al., 2011).

iv. *Cucumber vein yellowing virus*

Cucumber vein yellowing virus (CVYV; genus *Ipomovirus,* family *Potyviridae)* was reported in Lebanon, in 2013, infecting cucumber plants (Abrahamian et al., 2*013).* The virus is transmitted, in a semi-persistent manner, by the sweet potato whitefly, *B. tabaci*. The pathogen causes growth stunting and vein yellowing of the upper leaves causing significant yield reduction. Management of CVYV relies on growing tolerant plant varieties and controlling the vector by chemical, biological, and physical means (Gil-Salas et al., 2007).

Table 3. Summary of major viral diseases infecting cucumber plants in Lebanon

Viral disease	Causal agent	Genus	Family	Mode of transmission	Symptoms & Damage	Management
Cucurbit yellow stunting disorder disease	*Cucurbit yellow stunting disorder virus* (CYSDV)	*Crinivirus*	*Closteroviridae*	*B. tabaci*; semi-persistent manner	Interveinal chlorosis; thickness and brittleness of older leaves; plant growth stunting	Resistant/tolerant plant varieties; control of viral vector
Zucchini yellow mosaic disease	*Zucchini yellow mosaic virus* (ZYMV)	*Potyvirus*	*Potyviridae*	*M. persicae* & *A. gossypii* or infected seeds; non-circulative manner	Chlorotic mottling; deformation of leaves and fruits; plant stunting	Cross-protection with a mild strain; control of vector
Cucurbit chlorotic yellows disease	*Cucurbit chlorotic yellows virus* (CCYV)	*Crinivirus*	*Closteroviridae*	*B. tabaci*; semi-persistent manner	Yellow leaf spots; yellowing of lower leaves	Control of vector
Cucumber vein yellowing disease	*Cucumber vein yellowing virus* (CVYV)	*Ipomovirus*	*Potyviridae*	*B. tabaci;* semi-persistent manner	Growth stunting; yellowing of the upper leaves veins	Tolerant plant varieties; control of vector

3. *Integrated pest management*

Reliance on chemicals for pest control has simplified plant protection strategies and somewhat been effective in increasing crop yield. However, intensive use of pesticides has initiated severe human health and environmental problems along with the development of pest resistance (Barzman et al., 2015). Therefore, farmers are encouraged to adopt IPM strategies to avoid the adverse effects imposed by pesticides overuse (Kabir & Rainis, 2015).

According to the “European Union Framework Directive on the Sustainable Use of Pesticides”, IPM refers to the implementation of several control practices in order to suppress pest population below ETL, taking into consideration environmental and human health (Barzman et al., 2015). Since 1960-70s, IPM has been recognized as the dominant paradigm for crop protection (Parsa et al., 2014; Vreysen et al., 2007). General IPM principles include: preventing and suppressing pests; scouting/monitoring of harmful organisms; relying on cultural, physical, mechanical and biological control measures in preference to chemical measures; application of target-specific pesticides; limiting the use of synthetic chemicals; implementing anti-resistant strategies like applying pesticides having different modes of action; and record keeping (Barzman et al., 2015).

Biological control, an eco-friendly pest management measure, is an essential component of IPM (Naranjo et al., 2015) and it refers to suppression of harmful pest population by using beneficial organisms. Biological control has been practiced for more than 2,000 years; however its recent application began in the late 19th century. This control measure can be divided into four different categories, namely: conservation, natural, classical, and augmentative. In the latter type, natural enemies, including microbial organisms (bacteria and fungi) and invertebrates (predators and parasitoids) are reared and released in large amounts for rapid and effective control of pest infestations. Currently, there are around 350 commercially available invertebrate organisms and 94 different microbial species (almost 209 strains) (Van Lenteren et al., 2018). Hymenopteran parasitoids represent the largest group of invertebrates used in biological control systems followed by acarid predators. In biologically-based IPM systems, predators including *Neoseiulus cucumeris* Oudemans, *Amblyseius swirskii* Athias-Henriot and *Phytoseiulus persimilis* Athias-Henriot are considered the back-bone control agents. However, in order to have successful pest control in greenhouses, several compatible biological control agents should be released. An example of incompatibility is the case of *A. swirskii* which preys on eggs of *Aphidoletes aphidimyza* Rondani, a parasitic wasp of aphids (Buitenhuis et al., 2015).

Several success stories of biological control agents have been reported worldwide. For instance in Spain, sweet pepper farmers have relied completely on the predatory mite, *A. swirskii,* for the control of whiteflies and thrips (Calvo et al., 2015). Another success story includes the potential of *Nesidiocoris tenuis* Reuter, a hemipteran predator, to control *Tuta absoluta* on tomatoes (Mollá et al., 2014). *Bacillus thuringiensis,* a microbial organism used to control several harmful invertebrates on high-value agricultural crops, is another example of successful biological control agents (de Maagd, 2015).

a. Biological control agents for cucumber pests

i. *Phytoseiulus persimilis*

Phytoseiulus persimilis (Acari: Phytoseiidae) is a predatory mite used to effectively control spider mite species, mainly *T. urticae,* on vegetables and ornamentals (Ghazy et al., 2014). The life cycle of *P. persimilis* consists of 5 developmental stages, namely: egg, larva, two nymphal stages, and adult stage. The long-legged predatory mite is orange-red in color and more mobile than its prey, *T. urticae* (Malais & Ravensberg, 2004).

Population growth of the invertebrate organism and its pest control potential are highly dependent on environmental conditions, mainly temperature and RH (Stenseth, 1979). For instance, at temperatures ranging between 15ºC and 25ºC, *P. persimilis* population increases rapidly due to shorter generation time, higher fecundity rate, and larger female/male ratio. On the other hand, its activity decreases at temperatures exceeding 30ºC with RH less than 60%. Under optimal conditions, the predatory mite adult may consume up to 20 larvae, 20 eggs, or 5 adult spider mites each day. However, in the absence of prey, they may practice cannibalism (Malais & Ravensberg, 2004). Based on manufacturer's recommendation, approximately 12.5 *P. persimilis* adults/m^2 must be released on hotspots (Biobest©, Belgium). At severe spider mite infestation, selective acaricides must be applied; including hexythiazox, fenbutatin oxide, and clofentezin, for successful mite control. Effective pest control has been accomplished on cucumber crops but not on tomatoes due to sticky and poisonous stem and leaf hairs of the latter crop (Drukker et al., 1997). However, tomato conditioned *P. persimilis* are able to tolerate its secreted toxins and thus, establish and develop faster on tomato crop than the standard predatory mite (Drukker et al., 1997).

ii. *Amblyseius swirskii*

Amblyseius swirskii (Acari: Phytoseiidae) is a predatory mite used to control mainly larval stages of *F. occidentalis*, *T. tabaci*, and *Scirtothrips dorsalis* Hood and egg and nymphal stages of *B. tabaci* and *T. vaporariorum.* Being a generalist predator, *A. swirskii* can also feed on spider mite, broad mites, and eriophyid mite eggs (Buitenhuis et al., 2015). However, in the absence of prey, pollen of *Aesculus hippocastanum*, *Crocus vernus*, *Echinocereus* sp. and *Paulownia tomentosa* can act as a supplementary food source to maintain *A. swirskii* population *(*Goleva & Zebitz, 2013).

The predatory mite is used on wide range of greenhouse vegetables and ornamentals including chrysanthemums, gerberas, roses and poinsettia. Since *A. swirskii* has originated from the Mediterranean region, it is adapted to RH above 60 % and temperatures of up to 37ºC (Buitenhuis et al., 2015), but for optimal growth rate, temperatures ranging from 20ºC to 32ºC are the most favorable (Lee & Gillespie, 2011).

There are several commercially available formulations of *A. swirskii* products, including loose and slow release sachets. Slow release sachets are hanged on plants to ensure a continuous release of significant numbers of the predatory mites for a certain period of time, while loose formulations are sprinkled over plants ensuring easy movement of the biological control agent and efficient product usage (Buitenhuis et al., 2015). The recommended application rate of *A. swirskii* is 1 sachet/2m^2 (250 predatory mites in each sachet) (Biobest©, Belgium). Although a successful biological control agent, its introduction should be assisted by compatible synthetic pesticides; pymetrozine, flonicamid, and microbial pesticides like *Beauveria bassiana*, when pest infestation is high (Buitenhuis et al., 2015).

iii. *Eretmocerus eremicus*

Eretmocerus eremicus Rose & Zolnerowich (Hymenoptera: Aphelinidae) is an ecto-parasitic wasp used for the control of *T. vaporariorum* and *Bemisia* spp. (Luczynski et al., 2007). Adult wasps lay eggs under whitefly nymphs, preferably 2nd and third instars. Once the egg hatches, parasitoid larvae penetrate its host and start feeding on the haemolymph, leading to mortality of infested whitefly nymphs (Hoddle et al., 1998). Adult wasps emerge from parasitized whitefly pupae through a round exit hole (Biobest technical sheet). An adult female can lay eggs to parasitize almost 150 whitefly instars during its life span (Biobest©, Belgium). It is considered an effective biological control agent due to its ability to tolerate temperatures exceeding 30ºC, compatibility with several chemical insecticides, and high fecundity rate (Malais & Ravensberg, 2004). At temperature exceeding 20ºC and based on manufacturer's recommendation, weekly introduction of six parasitic wasps /m^2 should be employed (Biobest©, Belgium).

iv. *Aphidius colemani*

Aphidius colemani Viereck (Hymenoptera: Barconidae) is parasitic wasp belonging to sub-order aphidiinae, which entirely consists of aphid parasitoids. Along with *Aphidius matricariae* Haliday, *A. colemani* (most probably originating from India) is considered as a successful invertebrate organism used to control several aphid species, including *A. gossypii* and *M. persicae* (Zamani et al., 2007). A female wasp adult parasitizes its host through ovipositing an egg inside its prey. Once the egg hatches, emerged larva starts feeding on the parasitized aphid. As a consequence, the aphid dies and its body develops a crusty golden-brown shell, known as mummy. When *A. colemani* matures, it emerges through a round exit hole made on parasitized aphid (Stara et al., 2011). An adult female can lay eggs to parasitize more than 100 aphids within few days (Malais & Ravensberg, 2004). However, parasitism and development of *A. colemani* is dependent on several factors, mainly temperature. For instance, temperature ranging between 25°C and 30°C is optimal for high parasitism and rapid development rates of the parasitic wasp (Zamani et al., 2007). Based on manufacturer's recommendations, a release rate of 0.15 aphid mummies/m^2, on a weekly basis, is recommended as preventative introductions. The amount of beneficial organism is increased to 0.5-1 aphid mummy/m^2 upon detection of aphids on sticky traps (Biobest©, Belgium). *A. colemani* is successful in reducing aphid infestation. However, assistance with synthetic insecticides is needed when aphid population is too high. For instance, indoxacarb and methoxyfenozide could be suggested for that purpose due to their low toxicity on the natural enemy (Stara et al., 2011).

Table 4. Summary of successful biological control agents against major vegetable arthropod pests

Biological control agent	**Family**	**Prey/parasitize**	**Optimal T (ºC)**	**Prey consumption/ parasitism rate**	**Recommended rate**	**Compatible pesticides/bio-pesticides**
Phytoseiulus persimilis (Predatory mite)	Phytoseiidae	Spider-mites	15 ºC - 25 ºC	20 eggs, 20 larvae, or 5 adult spider-mites per day	12.5 *P. persimilis*/m^2	Hexythiazox; fenbutatin oxide; clofentezin
Amblyseius swirskii (Predatory mite)	Phytoseiidae	Thrips & whiteflies	20 ºC – 32 ºC	Not determined	125 *A. swirskii*/m^2	Pymetrozine; flonicamid; *Beauveria bassiana*
Eretmocerus eremicus (Parasitic wasp)	Aphelinidae	Whiteflies	20 ºC - 25 ºC	150 whitefly instars	6 *E. eremicus* /m^2/week	Buprofezin
Aphidius colemani (Parasitic wasp)	Barconidae	Aphids	25 °C - 30 °C	100 aphids within few days	0.15 aphid mummies/m^2/ week	Indoxacarb; methoxyfenozide

b. Banker/Habitat plants

Flowering plants may be used to attract and establish beneficial organisms in greenhouse before pest infestation, leading to more efficient pest control. As an example, ornamental peppers in their flowering stage can help *A. swirskii* to establish and effectively control whiteflies (Buitenhuis et al., 2015). Marigold is another example of flowering plants used to attract natural enemies like *Orius spp.*. Marigolds are available in the Lebanese market, easily grown and produce pollen which act as alternative food source for the beneficial organisms (Silveira et al., 2009).

C. Tomato production in Lebanon

Tomato, belonging to solanaceae family, is the most commonly cultivated vegetable worldwide. They are either consumed fresh or through processed food (Heuvelink, 2005). Solanaceae family encompasses many other important crops like potato (*Solanum tuberosum* L.), pepper (*Capsicum spp.)*, eggplant (*Solanum melongena* L.), and tobacco (*Nicotiana tabacum* L.) (Bosland, 1992; Heuvelink, 2005). Tomatoes are perennial vegetables; however, in temperate regions they are grown as annual crops due to sensitivity to temperatures below 12°C (Heuvelink, 2005; Peet & Welles, 2005). They are moderately salt tolerant; however, high salt concentrations may cause significant reduction in tomato fruit quality and yield, thus leading to considerable economical losses (Heuvelink, 2005; Li, 2000).

According to the Food and Agriculture Organization of the United Nations (FAO), world total tomato production recorded in 2016 reached 177 M tonnes on a surface cultivated area of 4.8 M Ha. In Lebanon, tomato recorded the highest production among other cultivated vegetables with 291,256 tonnes on a total harvested area of 3,566 Ha (FAOSTAT, 2016).

1. *Tuta absoluta, a major tomato insect pest*

Tuta absoluta (Lepidoptera: Gelechiidae) is one of the most harmful agricultural pests threatening tomato production in both; open field and protected cultivation systems. The tomato leaf miner, native to South America, was first reported in Spain in 2006. After its first detection, it has spread rapidly all over Europe and Mediterranean region. The host range of the gelechiid moth is restricted mainly to crops in Solanaceae family including cultivated and non-cultivated plants, along with some alternative plant hosts like *Datura stramonium* L. *and Physalis peruviana* L. Tomato is considered as the primary host of *T. absoluta* followed by other solanaceous crops like potato (*S. tuberosum* L.*)*, eggplant (*Solanum melongena* L.*)*, tobacco (*Nicotiana tabacum* L.*)*, and sweet pepper (*S. muricatum* L.) (Desneux et al., 2010).

Tuta absoluta is a multivoltine pest; it may complete up to 12 generations per year, depending on environmental conditions, mainly temperaure. For instance, development time decreases from aproximately 76 days at 14°C reaching a minimum of 23.8 days at 27.1°C. The average threshold of development for egg–larva–adult is 8.1 ± 0.2^{o}C. A total of 453.6 ± 3.9 DD is required for the moth to complete one generation. Under optimal conditions, a female can lay up to 260 eggs mostly on young leaves (73%) followed by oviposition on stem and leaf veins (21%) and, to a lesser extent, on sepals and fruits (Desneux et al., 2010; Tropea-Garzia et al., 2012). Eggs are usually laid singly on tomato leaves and stems. Once deposited, the eggs appear white to yellowish in color and during later stages they become coppery-red (Torres et al., 2001). Once hatched, the 1st instar larva remain outside the leaf for 5-40 minutes after which it starts boring into the leaf and feeding. After maturation the 4th instar larva pupates on the leaves or soil surface and emerges after about 5 days as adult moths (Luna et al., 2012; Tropea-Garzia et al., 2012).

Damage inflicted by *T. absoluta* occurs mainly at its larval stages through mining in leaves and feeding on photosynthetically active material, damaging tomato stems and buds, and burrowing galleries in fruits resulting in significant yield losses which could reach up to 100%, if not effectively treated (Cocco et al., 2013; Klieber & Reineke, 2016).

2. ***Common control methods of T. absoluta***

Control of *Tuta absoluta* is highly dependent on the use of synthetic insecticides to keep the population below ETL. In late 20th century, several pesticides have been proved to be effective for *T. absoluta* control including cartap, spinosad, chlorfenapyr, tebufonozide, abamectin (Desneux et al., 2010), methamidophos, permethrin, and deltamethrin (Guedes & Picanço, 2012). However, development of resistance against insecticides has been reported in several countries due to improper application of pesticides (Desneux et al., 2010; Guedes & Picanço, 2012). For instance, resistance to abamectin was reported in Brazil (Siqueira et al., 2001) along with methamidophos, permethrin and cartap (Siqueira et al., 2000). Moreover, resistance to deltamethrin in Argentina was also observed (Lietti et al., 2005). Therefore, reduction in pesticide use and alternation of insecticides with different modes of action should be implemented by farmers to lessen the high risk of developing resistance among pests (Guedes & Picanço, 2012) along with the reliance on other control practices to effectively manage pest infestation, like implementing biological control methods (Urbaneja et al., 2012).

Several biological control agents including invertebrates and microbial organisms have been evaluated for their efficacy in reducing *T. absoluta* population to economically acceptable levels. For instance, well established *Macrolophus pygmaeus* Rambour and *N. tenuis;* two mirid bugs preying on egg and larval stages of *T. absoluta,* were found to be successful in decreasing significantly leaf and fruit damages and thus considered as effective biological control agents *(*Desneux et al., 2010). However, in the absence of alternative food sources, *M. pygmaeus* becomes less efficient in managing pest population (Mollá et al., 2014). Moreover, Cabello et al., (2009) showed, under laboratory and greenhouse conditions, that *Trichogramma achaeae*, an egg parasitoid, can reduce *T. absoluta* damage by up to 91%. In Spain, *B. thuringiensis*, a commercially available microbial organism, showed high efficacy in controlling *T. absoluta* population, particularly the first larval instar (González-Cabrera et al., 2011). However, low level of resistance to *B. thuringiensis* was reported in Brazil (Silva et al., 2011). The use of entomopathogenic fungi for insect management is considered relatively safe to non-target organisms and has low probability of developing resistance under field conditions (Dubovskiy et al., 2013). Therefore, this may represent an eco-friendly approach for insect management by reducing the use of synthetic toxic pesticides. Over the years, several entomopathogenic fungi have been used for the control of several insect pests. Recently *Metarhizium anisopliae* and *B. bassiana* showed an effect on the larval stages of *T. absoluta* (Contreras et al., 2014; Klieber & Reineke, 2016; Shalaby et al., 2013) and on the egg stage (Pires et al., 2009; Shalaby et al., 2013).

3. *Beauveria spp. as a potential biological control agent against T. absoluta*

a. General overview of genus *Beauveria*

Beauveria sp., a facultative parasite well adapted to a saprophytic existence, is an entomopathogenic soil-borne fungus (Evans, 1982). This myco-parasite may also act as a plant endophyte (Vega et al., 2009). It was first demonstrated by Agostino Bassi to be the causal agent of white muscardine disease infecting silkworms (Rehner & Buckley, 2005). After its discovery, intensive research on the application of *Beauveria* spp. as an insect biological control agent was conducted by scientists for several compelling reasons. First, it is highly abundant and widely distributed in nature and easily recognized. Second, it has a wide host range (infecting more than 700 insect species) and broad variation in virulence towards different pests. Finally, it could be easily isolated from soil or infected insects, cultured on simple media, produced in massive quantities, and stored for a long period of time (Vega & Blackwell, 2005).

b. Classification of *Beauveria* spp.

Beauveria was assumed to exclusively have an asexual type of reproduction; by means of conidia (Vega & Blackwell, 2005). Thus, it was classified under phylum Deuteromycota; class Hyphomycetes, order Moniliales, and family Clavicipitaceae (Boucias & Pendland, 2012). However, recent advancements in molecular techniques demonstrated that *Beauveria* can undergo sexual reproduction and thus classified under phylum Ascomycota; class: Sordariomycetes; order: Hypocreales; family: Cordycipitaceae (Rehner et al., 2011).

At present, several *Beauveria* spp. are identified based on differences at their morphological and molecular levels; including *B. amorpha, B. australis, B. bassiana, B. pseudobassiana, B. brongniartii,* and *B. caledonica. B. bassiana* and *B. brongniartii* are used as biological control agents to manage pest infestation on high-value agricultural crops due to their myco-insecticidal characteristics (Rehner et al., 2011).

c. Infection mechanism

In general, *Beauveria* sp. infects its hosts through contact with the insect's exoskeleton. Once the fungal spore attaches to the external integument of the host, it germinates and enters through insect cuticle. For a successful invasion, several fungal enzymes (like proteases, lipoprotein lipases, chitinase, and amylase) must be secreted to degrade obstructing components of the insect cuticle, thus allowing easy penetration. After that, the host becomes prone to fungal secretion of toxic compounds (like Beauvericin and Bassianolide), invasion of its organs, and/or depletion of its nutrients (Feng et al., 1994). As a consequence, the infected host stops feeding, becomes weak and disoriented, and progressively changes its body color and appear like a mummy. With time, the infected host dies and under suitable environmental conditions, white powdery mycelial growth develops on its cadaver, fungal spores are produced and disperse by wind and water and infect new hosts by contact action (Poinar, 2012). Infection may also occur when the insect host ingests fungal spores while feeding on plants (Abdo, 2004).

Infection severity is dependent on several factors including host developmental stage, plant type, and environmental conditions. Insects in early developmental stages are more susceptible to fungal infection than those in advanced stages. Moreover, certain plants may secrete chemical compounds that could inhibit fungal germination and growth. Environmental conditions also play a critical role in determining fungal germination and growth rate and infection severity. In general, most fungi require warm temperature and RH exceeding 90% for optimal activity (Abdo, 2004). As an example, *B. bassiana* requires temperature ranging between 25°C -30°C and RH of 100% for optimal germination, growth, and sporulation (Walstad et al., 1970).

d. Isolation of *Beauveria*

Beauveria can be isolated from infected cadaver, on which fungal sporulation took place, and cultured on Sabouraud's Dextrose Agar (SDA), and then sub-cultured on PDA agar to obtain pure colonies (Abdo et al., 2008). In this technique, cadaver may be placed in a humid environment to hasten fungal sporulation and production of mycelia (Abdo, 2004). Recent isolation techniques include the exposure of the third larval instar of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) to field soil placed in a container. The container is then held in an incubator for 7 days at a temperature of 27°C. After which, the infected larvae is placed in a humid chamber for a period of 2 weeks. The mycelium that has developed on the insect cadaver is then transferred to the culture medium (Clifton et al., 2015). Another isolation method includes the inoculation of soil suspension onto PDA plates amended with 0.2 g alficetin, 0.2 g actinone, and 0.0133 g rose-bengal. The plates are then incubated at 25°C for a period of up to 7 days. After which, fungal colonies are cultured on PDA plates (Dong et al., 2016).

e. <u>Local *Beauveria* isolate</u>

A naturally occurring entomopathogenic fungus of the genus *Beauveria* was first reported in Lebanon infecting third larval instar of the cedar web-spinning sawfly, *Cephalcia tannourinensis* Chevin (Hymenoptera: Pamphiliidae) in Tannourine-Hadath El-Jebbeh cedar forest (Abdo et al., 2008). The Lebanese *Beauveria* isolate was first classified according to its conidial shape, which showed that its specie fits between *B. bassiana* clade C and *B. brongniartii* clade B. However for accurate identification, sequencing of nitrogen reductase, DNA lyase, and EF-1 genes along with the Internal Transcribed Spacer (ITS) regions were performed. Gene sequencing results showed that the local isolate is closely related to *Beauveria* cf. *Bassiana* Clade C (Abdo et al., 2008; Abou-Jawdah et al., 2008), now referred to as *B. pseudobassiana* (Rehner et al., 2011).

Beauveria pseudobassiana is phenotipically similar to *B. bassiana,* with the exception of conidial size. The surface mycelium of *B. pseudobassiana* is cottony in texture with white colony margin and interior. Conidia are 2-3 X 1.5-2.5 µm in size and primarily subglobose or broadly ellipsoid in shape. Its vegetative hyphae is septate, branched, and hyaline (Rehner et al., 2011). Germination and efficacy of the entomopathogenic fungi are affected by additives. For instance, corn oil affects positively the performance of the local isolate by increasing its germination rate by up to 70%. In contrast, pesticides like chlorphyriphos, malathion, endosulfan, monocrotophos, methyl parathion, and fenvelerate are incompatible with *Beauveria* due to their negative impact on the fungal germination rate (Baroudy, 2010). Previous study conducted by Abdo (2004) on evaluating the efficacy of the local *Beauveria* isolate against cedar web-spinning sawfly, *C. tannourinensis,* showed that both stages of the pest, egg and larvae, were infected by the fungus and mortality rates ranged between 85%-100% depending on conidial concentrations. Moreover, the experiment results proved that *B. pseudobassiana* treatment was more effective than diflubenzuron (Dimilin SC48), a reference insecticide used to successfully manage *C. tannourinensis* infestation (Abdo et al., 2008). Another study carried out by Abou-Jawdah et.al., (2008) showed, under laboratory conditions, that the local *Beauveria* isolate can successfully control the pine processionary moth, *Thaumetopoea wilkinsoni* Tams. Similar results were obtained when the pea leafminer, *Liriomyza huidobrensis* pupae was treated with the local *Beauveria* isolate (Noujeim et al., 2015).

The results of an experiment conducted by Klieber & Reineke, (2016) proved that *B. bassiana* could also be considered as an effective control treatment of *T. absoluta* due to its high virulence to all larval stages of the tomato leaf miner along with its significant effect on the hatchability of eggs. Moreover, Shalaby et al., (2013) showed that no egg hatching occurred when treated with the four conidial concentrations: 10^7, 10^8, 10^9 and 10^{10} conidia mL^{-1}. However, Inanli et al., (2012) reported that only 41.67% and 66.67% of eggs did not hatch.

CHAPTER III

MATERIALS AND METHODS

A. Evaluation of the efficacy of arthropod predators and parasites for the management of greenhouse cucumber pests

1. *Experimental Sites*

The experiments were conducted at two sites: Tamich (Site A), Mount-Lebanon, at an altitude of 250 meters and Zahrani (Site B), South of Lebanon, at an altitude of 5 meters. Two greenhouses were selected at each site. One greenhouse served as control, where the farmer followed his normal production and protection practices while in the second greenhouse, the IPM practices were implemented.

At site A, the area of each single span greenhouse was 414 m^2. The cocopeat-based hydroponic system was adopted in both greenhouses. The greenhouse's soil was covered with thick, opaque geotextile fabric to prevent weed germination. The transplanting of cucumber seedlings, variety New Sun, took place on the 3rd of November 2016, at a rate of 3 cucumber seedlings/m^2. At site B, the area of each single span greenhouse was 320 m^2 and in both greenhouses the cucumber seedlings, variety Mira, were transplanted on 19th of October 2016, at a rate of 3 seedlings/m^2.

2. *Pre-transplanting measures in IPM greenhouses*

All greenhouses were equipped with clean polyethylene covers and the vents were closed by insect-proof nets. Double doors were installed in the IPM greenhouses to reduce insect introductions. Data loggers (Ebro®) were installed to record daily temperature and RH, at an interval of 15 minutes. For the elimination of recently introduced whiteflies, thrips and fungus gnats from the IPM greenhouses, yellow sticky cards (YSCs) and blue sticky cards (BSCs); provided by the Ministry of Agriculture extension services, were installed at a rate of 1 trap/16m^2. About a week before transplanting, trap plants, in the form of blossomed marigold pots, were placed in the IPM greenhouses at a rate of 1 flowering plant per 32 m^2 and 41 m^2, respectively. Each pot contained 2 yellow marigolds (ferry-morse®) that were grown in a nursery and were not treated with any type of insecticide. Daily irrigation of the trap plants was accomplished through a watering can. One day prior to transplanting, marigolds were covered by a nylon bag and discarded to eliminate any insect that may have entered the greenhouse and was attracted to the trap plants. The removed marigolds were replaced by new ones. Weeds, inside and around the greenhouses were pulled by hands instead of applying herbicides and plant debris, left from last season, were removed and burned. At site A, coco-peat substrate was treated with hydrogen peroxide, which was applied through drip-irrigation system, to ensure the death of harmful microorganisms and insects while at site B, primary and secondary tillage were performed mainly to soften the soil and to reduce clod size.

3. *Pre-transplanting measures in control greenhouse*

In control greenhouses, same pre-transplanting practices, performed in IPM greenhouses, were followed except for the introduction of yellow/blue sticky traps and potted marigolds and the installation of double doors. In addition, chlorpyrifos (a broad-spectrum insecticide) was sprayed on the greenhouses polyethylene plastic cover in both locations.

4. *Post-transplanting measures*

a. Insect/mite scouting

Weekly insect/mite scouting was performed in both locations and in both greenhouses. A total of 50 randomly selected cucumber plants were monitored per greenhouse. From each selected plant, 3 leaves and 3 flowers from the top, middle and lower part of the crop were randomly chosen and scouted; that is a total of 150 leaves and 150 flowers per greenhouse. The number of whiteflies nymphs, whitefly adults, thrips, and mites, were recorded. The same scouting technique was used also for monitoring populations of the released natural enemies; *A. swirskii*, *P. presimilis*, and *E. eremicus*. Throughout the growing period, highly infested marigolds and cucumber leaves/plants were removed from the IPM greenhouses and burned. New flowering marigolds replaced the removed ones. Sticky traps were also replaced by new ones every two months.

b. Natural enemy introductions

The transportation cost of the natural enemies to Lebanon was considered very expensive. For that reason, it was preferred to perform the introductions based on monitoring of the arthropod pest populations and not more than one release per month. During the experimental period, four natural enemy introductions were performed at site A and five at site B. The beneficial organisms used were *A. swirskii* and *P. persimilis*. Nutrimite® was used as supplemental pollen food source for *A. swirskii* to improve its establishment rate (Table 5). *E. eremicus* was also imported several times, as parasitized whitefly nymphs, but the emergence rate was low and the efficiency of the emerged adults was minimal, since neither adult parasitic wasps were recorded on the cucumber plants nor parasitized whitefly instars were observed. No data are presented on the efficacy of *E. eremicus*.

Table 5. Dates of introduction and release rates of natural enemies in the IPM greenhouses, at sites A and B

	B		A	
Date	Natural enemy	Release Rate	Natural Enemy	Release Rate
11/4/2016	*A. swirskii* system bottle (25,000 mites)	78/m^2	-	
	Nutrimite®	0.15g/m^2		
11/22/2016	*A. swirskii* breeding system 500 (125,000 mites)	390/m^2	*A. swirskii* system bottle (25,000 mites)	60/m^2
	Nutrimite®	0.15g/m^2	Nutrimite®	0.12g/m^2
12/14/2016	*A. swirskii* breeding system 500 (125,000 mites)	390/m^2	*A. swirskii* breeding system 500 (125,000 mites)	300/m^2
	P. persimilis 2 bottles (2000 mite/bottle)	12.5/m^2	Nutrimite®	0.12g/m^2
			P. persimilis 2 bottles (2000 mite/bottle)	10/m^2
2/22/2017	*A. swirskii* breeding system 500 (125,000 mites)	390/m^2	-	
	P. persimilis 2 bottles (2000 mites/bottle)	12.5/m^2		
2/23/2017	-		*A. swirskii* breeding system 500 (125,000 mites)	300/m^2
			P. persimilis 2 bottles (2000 mites/bottle)	10/m^2
3/31/2017	*P. persimilis* 2 bottles (2000 mites/bottle)	12.5/m^2	*A. swirskii* system bottle (50,000 mite)	120/m^2
			P. persimilis 6 bottles (2000 mites/bottle)	29/m^2

c. Pesticide sprays

During the growing period, the IPM greenhouses required some insecticidal/acaricidal and fungicidal sprays to assist the natural enemies in reducing high arthropod pest infestations and to lower disease incidences. Most of the pesticides used in the IPM greenhouses were chosen based on IPM guidelines and natural enemy safety precautions. In the control greenhouses, the farmers conducted their normal pesticide sprays.

i. IPM greenhouses

For the IPM greenhouses, list of non-harmful pesticides to natural enemies of interest was prepared based on the side-effect manual data sets created by Koppert© and Biobest© groups (Appendix I, Table I).

At site B, only one miticide spray; cyflumetofen, was applied by the end of January while at least 3 safe listed fungicides (azoxystrobin, myclobutanil, and fenhexamid) and dimethomorph were applied as foliar spray for the control of powdery mildew, downy mildew, botrytis, and sclerotinia. Thiophanate-methyl and Fosethyl-aluminum were applied through the irrigation system for control of fungi. At site A, 3 miticide sprays; acequinocyl, bifenazate and spriodiclofen, were applied to reduce mite populations. The fungicides propamocarb hydrochloride, penconazole, kresoxim-methyl, and thiophanate-methyl were sprayed for downy and powdery mildew control. Details of pesticides applied in the IPM greenhouses, including rates and dates of application, active ingredients, trade names, and target pests, are provided in Appendix I, table II.

ii. Control greenhouses

At site B, at least 15 pesticide sprays were applied during the growing season, each consisting of a minimum of 3 pesticides. Even though the first recorded pesticide spray was on the 10th of January 2017, prior to the latter date several pesticide sprays were applied, roughly at weekly intervals, but not recorded by the farmer. Some applications contained up to 5 different pesticides in one spray. Main sprays were fungicides targeting downy and powdery mildews while the insecticides used were those targeting mites, thrips, and whiteflies. At site A, 11 pesticide sprays were applied but the farmer tried to use safer pesticides and refrained from mixing different types during the same application date. Details of pesticides applied in the control greenhouses, including rates and dates of application, active ingredients, trade names, and target pest, are provided in Appendix I, tables III&VI.

5. *Cucumber yield data*

At both experimental sites, cucumber yields produced in the IPM and control greenhouses were recorded until the last crop harvest performed in the control greenhouse. The yield data were collected from a total of 950 cucumber plants per greenhouse at site A and 900 plants per greenhouse at site B.

6. ***Statistical analysis***

Statistical analysis was performed using IBM SPSS statistics 24. Average number of insects and mites per leaf and thrips per flower of each plant was calculated. Statistical analysis was divided into the following parts:

1. The efficiency of pest control program adopted at each greenhouse (IPM strategy or conventional pest control strategy), in regards of all arthropod pests, was evaluated using two-way ANOVA at each location.
2. The efficiency of control of each beneficial organism to its corresponding pest was determined by comparing the population of each insect in the IPM greenhouse to its population in the control greenhouse, at each location, using two-way ANOVA.
3. The insects populations trapped on YSCs/BSCs and marigold flowers, in the IPM greenhouse, were compared to those attracted to cucumber leaves and/or flowers using two-way ANOVA

Since at sites A and B, the farmers had to stop the control greenhouses on 21/3/2017 and 17/3/2017 respectively, statistical analysis related to comparisons between greenhouses was conducted using the scouting data recorded until the last date of data collection in the control greenhouse, at each location.

B. Evaluation of the efficacy of *B. pseudobassiana* against *T. absoluta*, under laboratory conditions

1. *T. absoluta collection and rearing*

Tuta absoluta adults were collected, by a cordless vacuum cleaner (Bosch®), from tomato greenhouses located in Der-Tamich region, Mount of Lebanon. The insects were released and reared on potted tomato plants placed inside insect-proof cages (55 cm x 55 cm x 55 cm), in a greenhouse, at the American University of Beirut (AUB). Dead tomato plants were replaced by new, healthy ones to maintain *T. absoluta* population. Data logger (Ebro®) was placed in the greenhouse to record daily temperature and RH, at an interval of 15 minutes. Throughout the time of insect rearing, the RH ranged between 40-90% and temperature between 18-35°C.

2. *Beauveria spp. cultures used in bio-assays*

Long-term stored fungal cultures, covered with sterile 10% glycerol and incubated in eppendorf tubes at – 80°C, were re-activated by transferring fungal mycelia, under sterile conditions, with a plastic loop to Potato Dextrose Agar (HiMedia®) in a 9 cm Petri-dish and incubated at 25°C. In order to stimulate fast fungal growth, sub-culturing of the fungus, on new PDA plates, was performed every 4-5 days through mycelial transfer. The pure cultures were then maintained at 25°C on PDA plates and sub-cultured every 20-25 days.

3. ***Bioassays***

a. Preparation of conidial suspensions

Fungal conidial suspensions were prepared by adding 10 mL sterile distilled water with 0.01% Tween-20 (Sigma®) (as wetting dispersing agent) in each Petri dish containing two-week old *Beauveria* cultures. With a sterile metal rod, the fungal culture was gently rubbed, and the resultant suspension was filtered through three layers of cheesecloth. Conidia counts were performed using an improved Neubauer haemocytometer and diluted to the desired concentrations i.e. 10^6,10^7 and 10^8conidia mL^{-1}.

To try to improve the efficacy of control, just before spraying, the conidial suspensions were amended, in some treatments, either with corn oil at a rate of 1% or the commercial oil, Codacide®, at the recommended rate of 0.1%.

b. Preparation of plant material

Tomato seeds, variety B5 Baladi, were sown in 50 plug seed trays and placed in a growth chamber at 28°C, 80 to 85% RH, and 16:8 hrs L:D photoperiod. After three weeks, the seedlings, at two true-leaves stage, were transplanted in 15 cm diameter pots and placed, in the greenhouse, in insect-proof cages at a rate of 10 tomato seedlings per cage. A total of 40 *T. absoluta* adults were released per cage and left, for 48 hours, to lay eggs. Then the seedlings were removed for performing the bioassay experiments and replaced by new ones for maintaining the insect population.

c. Preliminary bio-assay method

Tuta absoluta eggs were placed on the lower side of detached tomato leaves, using a fine brush, at a rate of 5 eggs/leaf. Each leaf was placed inside a Petri dish containing moistened filter paper to increase the RH to 100%. The bio-assay was conducted with three different conidial concentrations; 10^6, 10^7 and 10^8 conidia mL^{-1}, amended with or without their respective adjuvant. In total, 13 different treatments, including four negative controls, were sprayed uniformly on the leaves using 50 ml spray bottles. All the treatments were replicated 6 times; a total of 30 eggs/treatment. List of applied treatments and number of *T. absoluta* eggs/replicate are provided in Table 6.

The Petri-dishes containing the sprayed leaves were sealed with Parafilm and incubated at 24°C with 100 % RH and 16:8 hrs L:D photoperiod. The eggs were monitored daily and data for cumulative mortality rates were recorded after 7 days of incubation.

For determination of the efficacy of control, data were collected on egg mortality and neonate larval mortality. Results were recorded as: sporulated/non-hatched eggs, non-sporulated/non-hatched eggs and hatched eggs. The larvae from the hatched eggs were monitored for mortality rate and recorded as either healthy/living when leaf mines were more than 5 mm long or dead when the mines were less than 5 mm long. In the latter case, the mine was opened, and the shriveled-dark brown larva was inspected. Some of these larvae were maintained in a humid chamber to observe whether fungal fructifications will appear. Corrected mortality (CM) was calculated using the Abbott formula (Abbott 1925).

$$\text{Corrected mortality \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) \times 100$$

Where: n = insect population, T = treated, Co = Control

d. In-vitro bio-assays, egg and larval susceptibility

As in the preliminary trial, *Tuta absoluta* eggs were placed on the lower side of detached tomato leaves, using a fine brush, at a rate of 6 eggs/leaf and placed inside petri dishes containing moistened filter paper. Based on results of the preliminary experiment, only the two conidial suspensions that gave the highest egg mortality; 10^7 and 10^8 conidia mL^{-1} amended with 1% corn oil, were included in this test, in addition to two negative controls namely: water and water + 0.01% tween-20 + 1% corn oil.

To compare the control efficacy of local *Beauveria* isolate with commonly used management methods, two additional treatments were included; abamectin (a.i. 1.8%, EW; DIVA®) at a rate of 0.5 mL L^{-1} and *B. thuringiensis* subsp. Kurstaki (32,000 spores/mg, WP; Javelin®) at a rate of $5.4x10^7$ spores L^{-1}.

In total, 4 different treatments and two negative controls were sprayed uniformly on the leaves using 50 ml spray bottles. All the treatments were replicated 7 times; a total of 42 eggs/treatment and the whole experiment was performed three times at roughly monthly intervals. List of applied treatments and number of *T. absoluta* eggs/replicate are provided in Table 7.

The same techniques used in the preliminary bio-assay, regarding incubation of tomato leaves and determination of the control efficacy of *Beauveria* on the egg stage of *T. absoluta*, were followed in these three experiments.

4. ***Statistical analysis***

A total number of 1,146 *T. absoluta* eggs were used to conduct the experiments. A regression analysis of the corresponding mortality was performed, using IBM SPSS statistics 24, determining a correlation between fungal efficacy and its conidia count. One-way ANOVA followed by LSD test and split plot analysis were performed on experimental data to determine the control efficacy of the treatments applied against *T. absoluta* eggs.

CHAPTER IV

RESULTS & DISCUSSION

A. Evaluation of the efficacy of arthropod predators and parasites for the management of greenhouse cucumber pests

1. *Temperature and RH variations recorded at the two experimental sites*

The data loggers, set in the IPM greenhouses, were of low manufacturing quality and non-functional during part of the growing period. The retrieved data is presented in Appendix II, figures I & II. At both locations, the temperature was inversely proportional to the RH. Throughout most of the experimental period, the temperature and RH recorded at site B were respectively around 4-5°C and 7% higher than that at site A. This is explained by the difference in altitude/elevation above sea level between the two experimental sites.

2. *Population dynamics of insects/mite in control and IPM greenhouses*

a. <u>Site A</u>

The population dynamics of whiteflies, thrips, and mites and their corresponding natural enemies, in the control and IPM greenhouses, are presented in Figures 1-6.

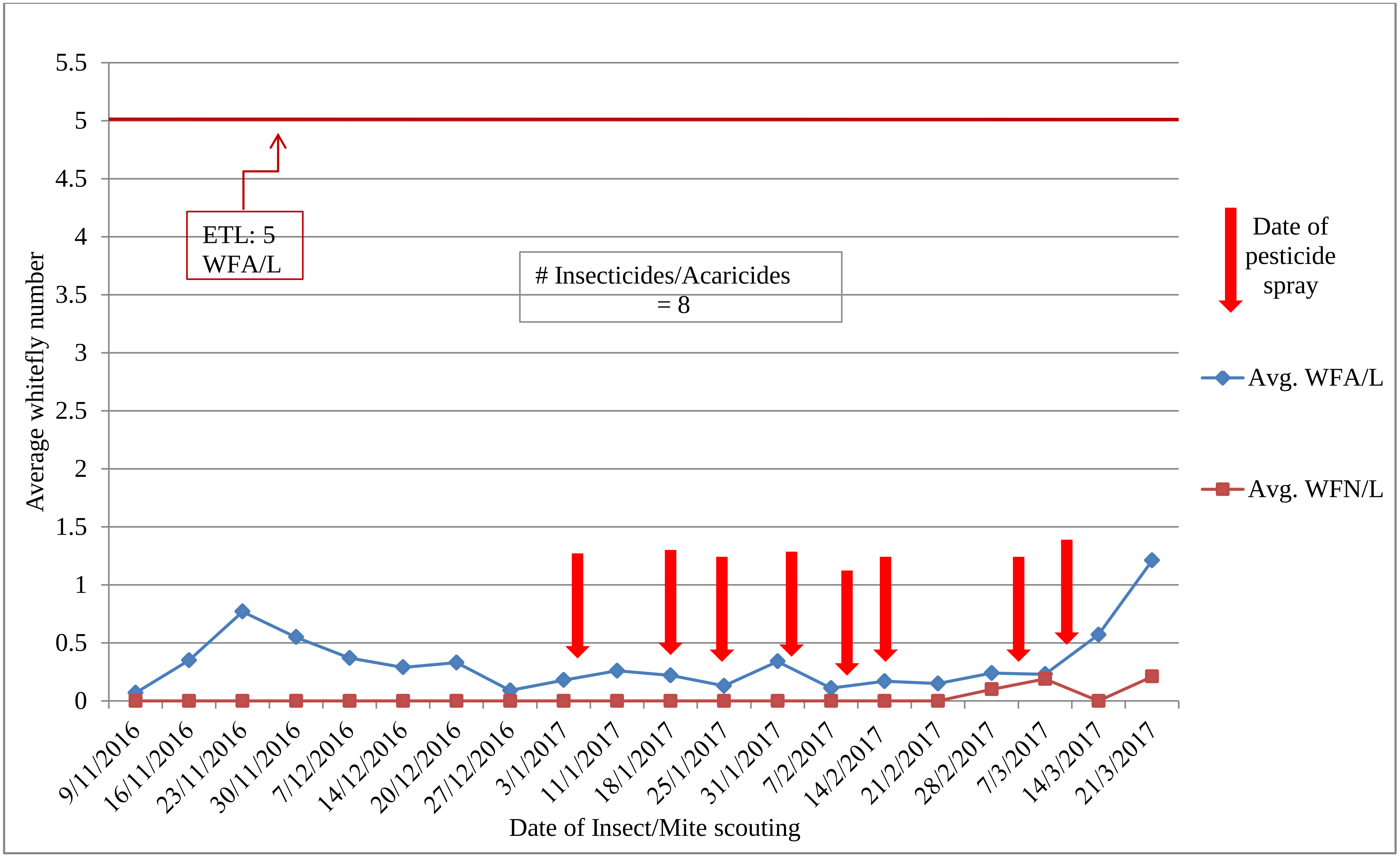


Figure 1. Average whitefly adult and nymph populations recorded on cucumber leaves throughout the growing period in the control greenhouse, at site A. A total of 8 insecticidal/acaricidal sprays were performed in the greenhouse. WF/L: Whitefly per Leaf; WFN/L: Whitefly nymph per Leaf; ETL: Economic threshold level

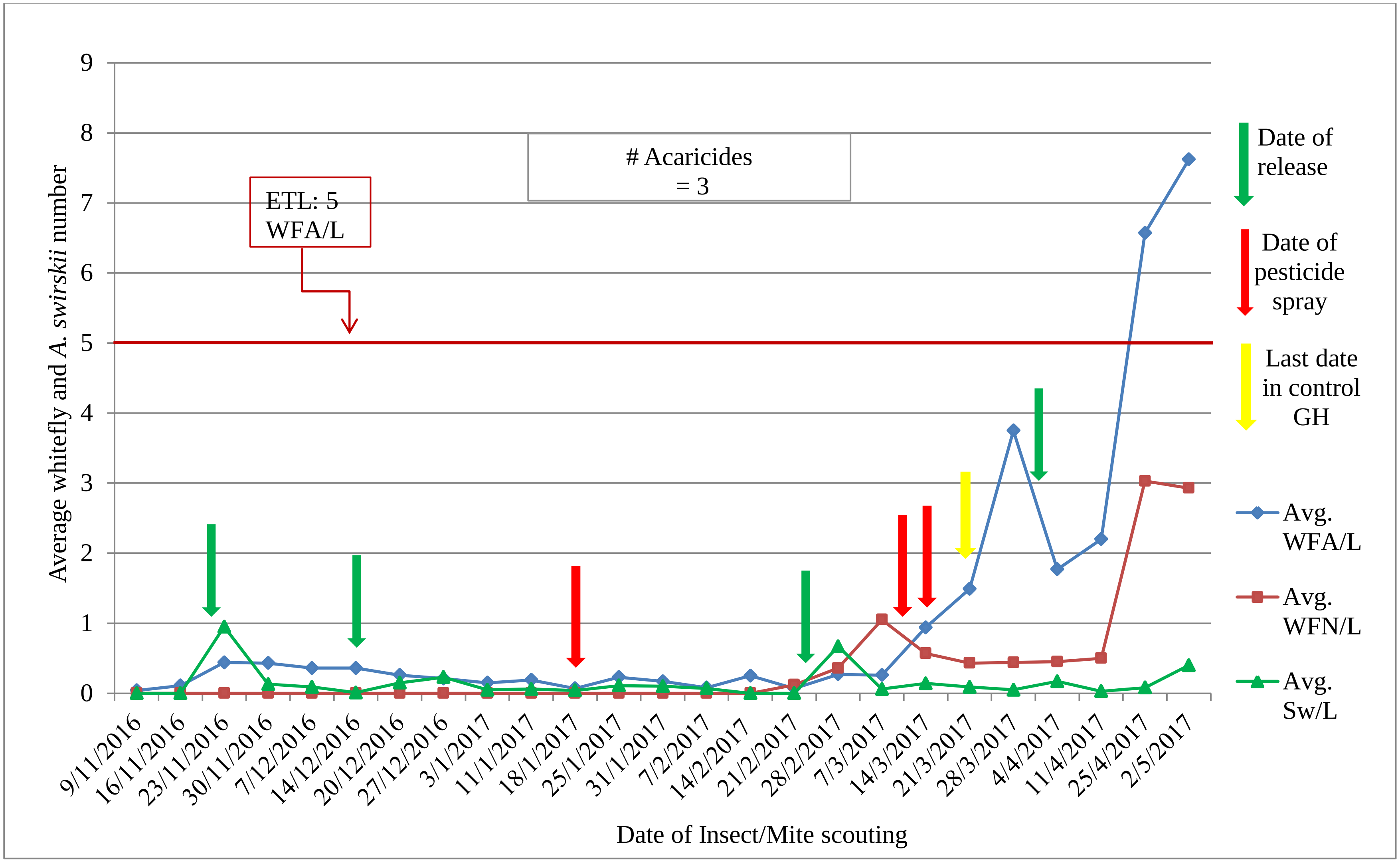


Figure 2. Average whitefly nymph and adult and *Amblyseius swirskii* populations recorded on cucumber leaves throughout the growing period in the IPM greenhouse, at site A. A total of 4 introductions of *A. swirskii* and 3 acaricidal sprays were performed in the greenhouse. WF/L: Whitefly per Leaf; WFN/Leaf: Whitefly nymph per leaf; Sw/L: *Amblyseius swirskii* per Leaf; ETL: Economic threshold level

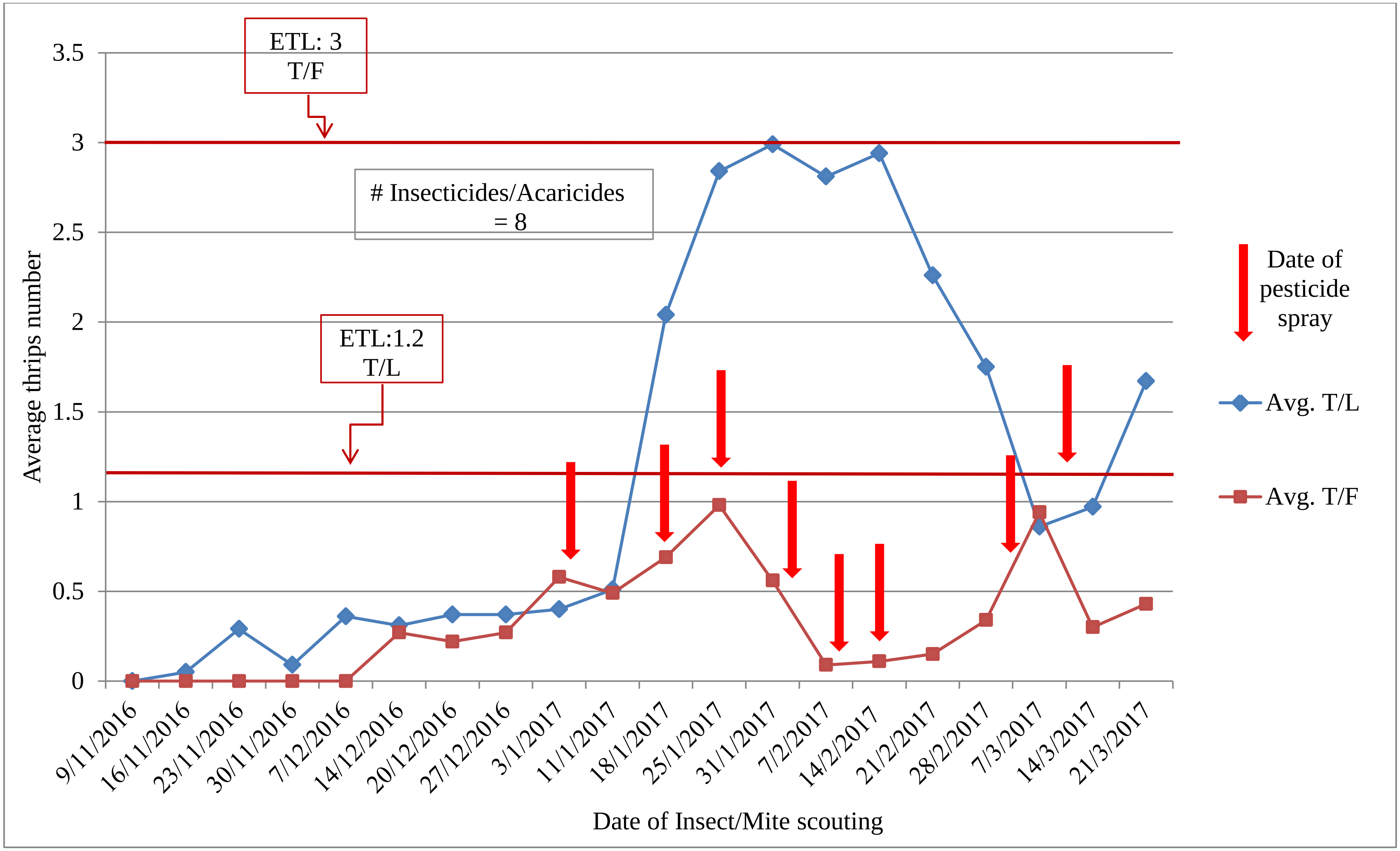


Figure 3. Average thrips population recorded on cucumber leaves and flowers throughout the growing period in the control greenhouse, at site A. A total of 8 insecticidal/acaricidal sprays were performed in the greenhouse. T/L: Thrips per leaf; T/F: Thrips per flower; ETL: Economic threshold level

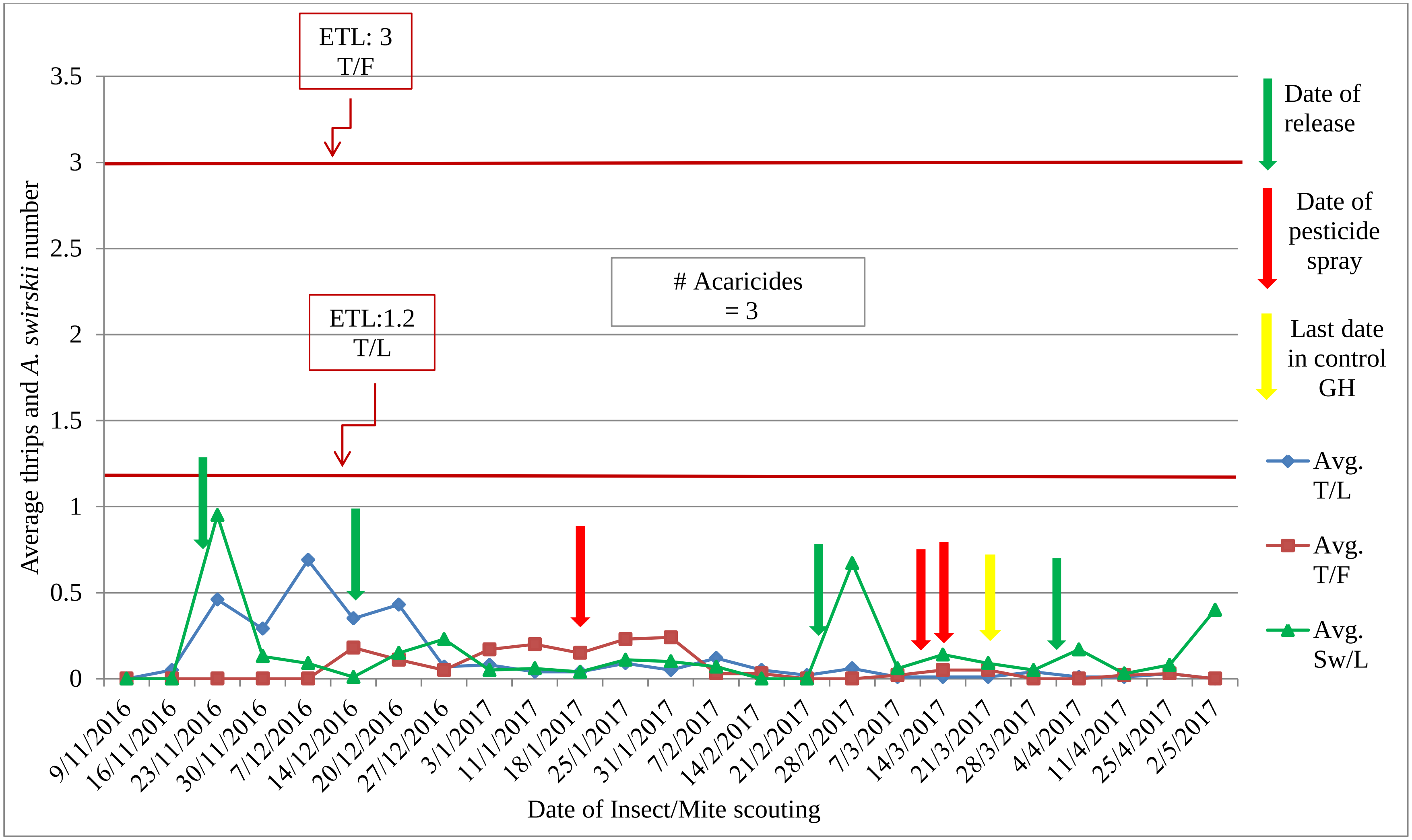


Figure 4. Average thrips populations recorded on cucumber flowers and leaves and average *Amblyseius swirskii* populations recorded on cucumber leaves throughout the growing period in the IPM greenhouse, at site A. A total of 4 introductions of *A. swirskii* and 3 acaricidal sprays were performed in the greenhouse. T/L: Thrips per leaf; T/F: Thrips per flower; Sw/L: *Amblyseius swirskii* per leaf; ETL: Economic threshold level

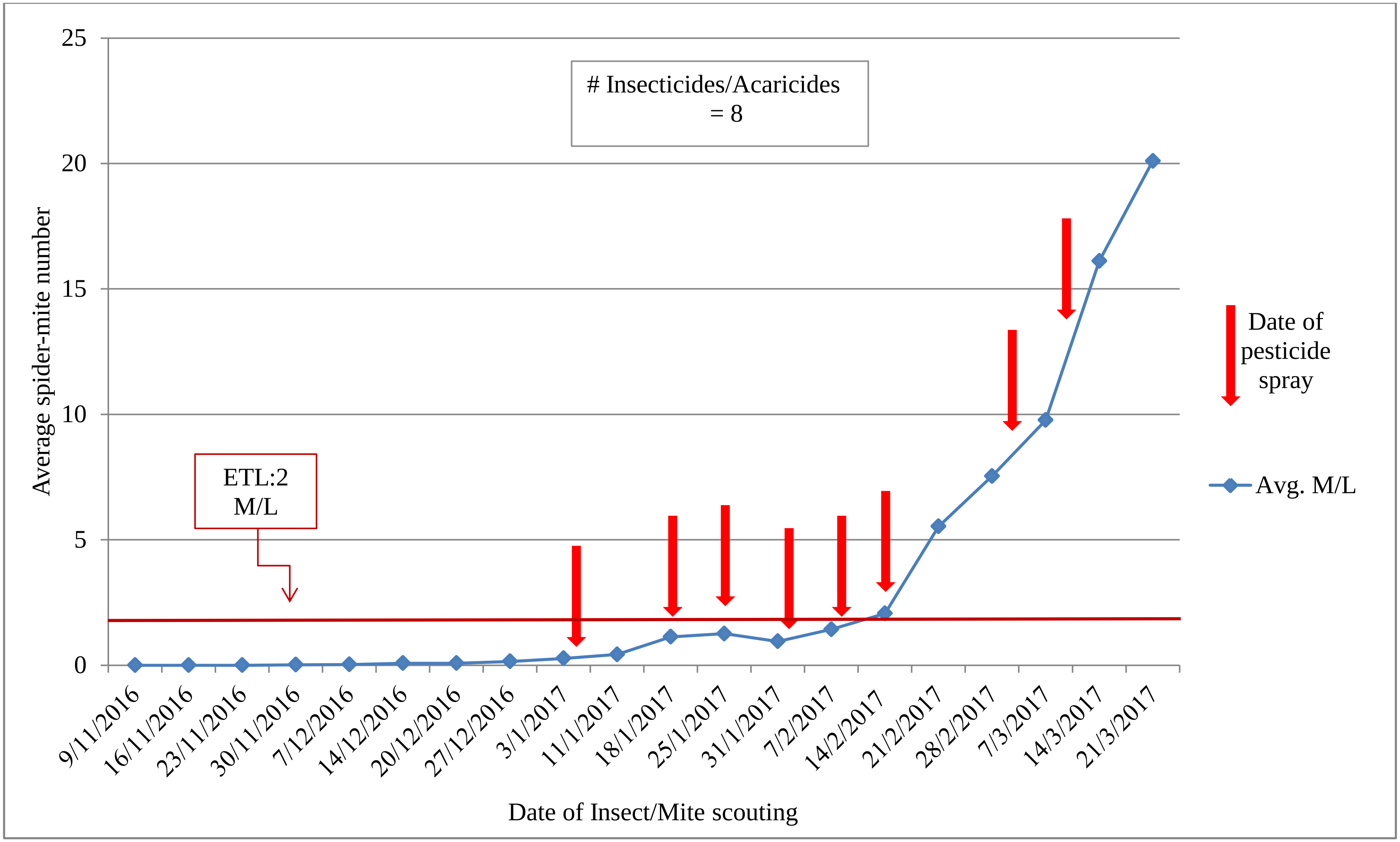


Figure 5. Average spider-mite population recorded on cucumber leaves throughout the growing period in the control greenhouse, at site A. A total of 8 insecticidal/acaricidal sprays were performed in the greenhouse. M/L: Spider-mites/Leaf; ETL: Economic threshold level

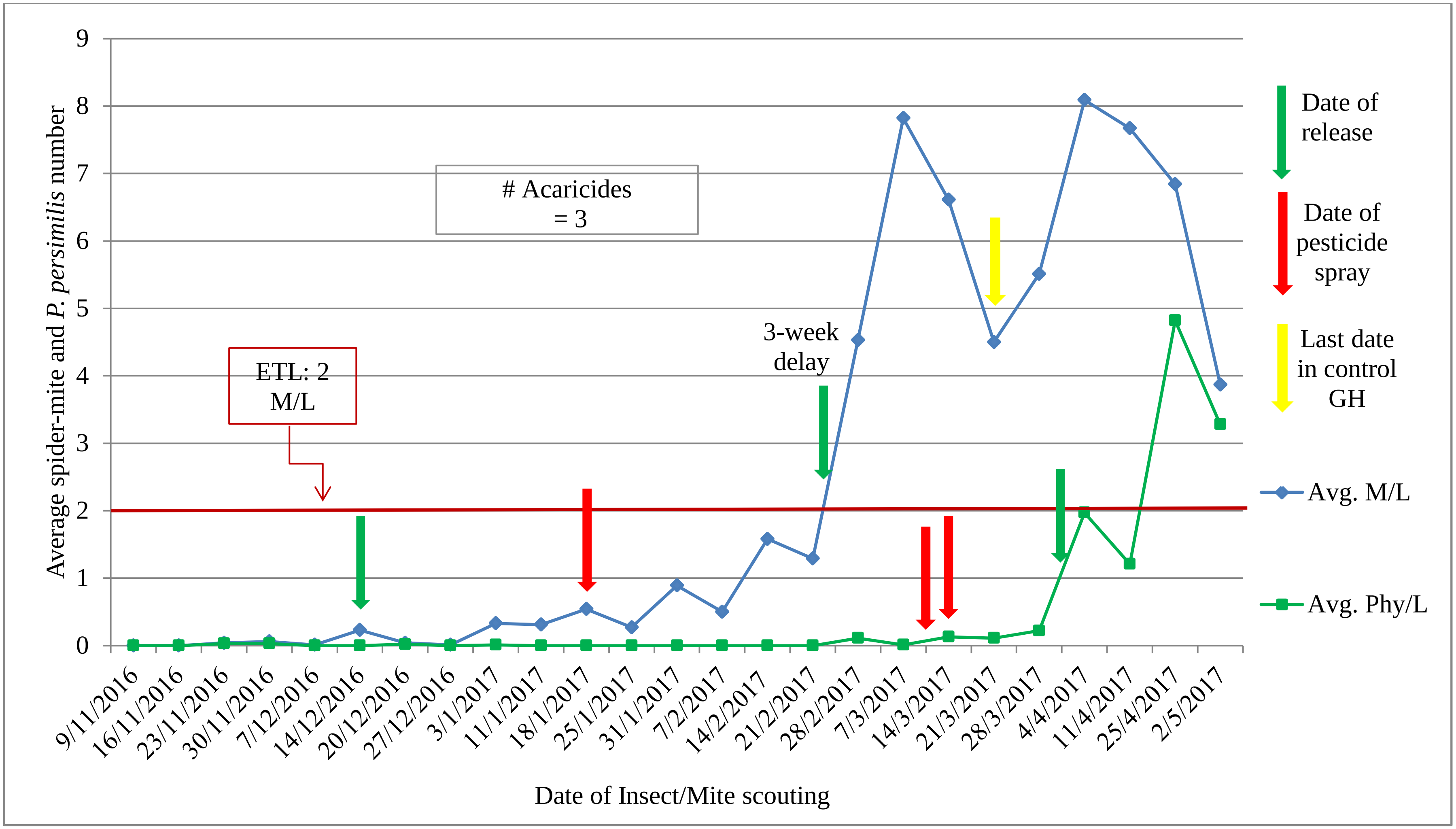


Figure 6. Average spider-mite and *Phytoseiulus persimilis* populations recorded on cucumber leaves throughout the growing period in the IPM greenhouse, at site A. A total of 3 introductions of *P. persimilis* and 3 acaricidal sprays were performed in the greenhouse. M/L: Spider-mites per Leaf; Phy/L: *Phytoseiulus persimilis* per leaf; ETL: Economic threshold level

In the control greenhouse, the farmer did not perform any insecticidal sprays during November and December since the insects (whiteflies and thrips) and mite populations were below a level of 1/leaf or flower. This corresponds to a period where the average temperature was below 15° C and the average maximum temperature was mostly below 20° C. This is in agreement with the results reported by Malais & Ravensberg, (2004) whereby it was shown that the development of whiteflies, thrips, and mites is mainly dependent on temperature and slows down significantly at an average temperature below 16° C. However, starting from the first week of January, the average temperature increased to above 15° C and the maximum temperature has most often exceeded 30° C, reaching 35° C (Appendix II, Figure I). This noticeable increase in temperature favored the development of the arthropod pests and thus prompted the farmer to start his first insecticidal/acaricidal sprays that totaled 8 applications during the entire growing season. Throughout the experimental period, whitefly population was maintained below ETL, with the highest peak of 1.21 adults and 0.21 nymphs per leaf recorded on the third week of March (Fig. 1). Similarly, thrips population recorded on cucumber flowers remained below damaging level (less than 1 thrips/flower). While, thrips population on cucumber leaves exceeded the EIL form mid-January till the end of February, reaching a maximum number of 2.99 thrips/ leaf (Fig. 3). These observations contradict the result stated by Malais & Ravensberg, 2004, whereby it was found that thrips are more abundant on cucumber flowers than on leaves since they undergo faster development rate on flowers. The high thrips population recorded on the leaves is mainly explained by minimal plant flowering rate during that period-low crop vigor and non-optimal fertilization management. As for mite population, it has increased rapidly starting from early February (1.5 mites/leaf), reaching over 20 mites per leaf by the third week of March (Fig. 5), despite of three acaricidal sprays (hexythiazox+ chlorfenapyr, abamectin, and bifenazate), forcing the farmer to remove the crop due to severe mite infestation (Fig. 7). The inefficacy of the mentioned acaricides may be due to several factors including; development of mite resistance, inappropriate application method or rate, and/or low quality of pesticide. In general, the farmer was environmentally aware and normally applied pesticides when deemed necessary however; he was not efficient in selecting the appropriate insecticides/acaricides that best fit his needs.

Figure 7. Cucumber plant showing high mite infestation in the control greenhouse, at site A. Spider mite webs and spotting of leaves are observed due to the very large number of *T. urticae*

In the IPM greenhouse, four natural enemy introductions were performed throughout the cucumber growing period while, insecticides were not used. The whitefly nymph and adult populations were maintained below ETL till Mid-April; less than 3.8 adults and 1.1 nymphs/ leaf. Although considerable whitefly adult migration into the greenhouse was recorded from mid-March till early May reaching a maximum peak of 7.62 adults/leaf, the last introduction of *A. swirskii* (at a rate of 120 predators/ m^2) was able to maintain the nymph population to below 3.1 nymphs (Fig. 2). These observations suggest that *A. swirskii* was effective in maintaining whitefly population despite of its low recorded population which was mainly due to low prey (thrips and whitefly nymphs) density and difficult movement of the predator from plant to plant (leaves were not in contact with each other). Similarly, thrips populations were maintained below 0.7 thrips per leaf/flower (Fig. 4) showing that *A. swirskii* was more successful than the insecticides used in the control greenhouse for thrips management. As for *T. urticae* population, Biobest© recommend releasing *P. persimilis* as soon as the first spider-mite hot spot is detected. Based on the company's recommendation, the first release of the predator was conducted on mid-December however; its population was not observed on the following weeks. As a consequence, *T. urticae* population had increased gradually till the end of January however, it remained below ETL (less than 1 mite/ leaf) and thus no predators were ordered in the latter month. After which, mite population increased significantly to reach about 8 mites/ leaf, in a period of 5 weeks. Accordingly, two consecutive sprays of specific acaricides; bifenazate and spirodiclofen, were performed. The control efficacy of the latter pesticides was limited since mite population was reduced to only about 5.5 mite/leaf within a period of two weeks. After which, the population increased. The high mite number along with the suitable temperature for *T. urticae* development prompted to triple the recommended rate of the predator (29 *P. persimilis*/m^2). This strategy proved effective and reduced mite population from around 8 to less than 4 mites per leaf within 3 weeks. This was associated with an increase in the number of predators which reached up to 4.9 predators/ leaf three weeks post introduction (Fig. 6), proving the high efficacy of the predator. The increase in *P. persimilis* population was mainly due its high quality, available prey and suitable environmental conditions. The population dynamic of *T. urticae* and *P. persimilis* was also reported in several studies. For instance, regarding the first predator release, Gontijo et al. (2010) showed that *P. persimilis* is not effective when released during very low level of mite infestation on crops with complex architecture like cucumbers. As for the considerable increase in mite population (8 mites/leaf), this was mainly due to a 3-week delay in the 2nd *P. persimilis* introduction which should have taken place in the beginning of February, low recorded predator population during that period, and favorable environmental conditions for mite development. In addition, the quality and number of the predator released (2nd introduction) were severely affected by a 24-hours delay in introduction mainly due to unfavorable storage conditions and/or a very short life expectancy of the predator (Biobest©, Belgium). Other scientists proved that *P. persimilis*, stored at a temperature around 20° C, undergo high rate of cannibalism when deprived from food for a period exceeding 12 hours (Sabelis, 1982). Concerning the high efficacy of the predator, similar results were reported by Opit et al. (2004) whereby *P. persimilis*, released at a ratio of 1:4 (predator : prey), has effectively controlled spider mite infestation on ivy geranium. Other studies showed the high efficacy of the predator on greenhouse grown strawberries (McMurtry et al., 1978).

Statistically, the averages insects/mite populations were compared using data gathered until the last day of data collection in control greenhouse (21/3/2017). The averages of whitefly, thrips, and mite populations in the IPM and control greenhouses are presented in Figure 8.

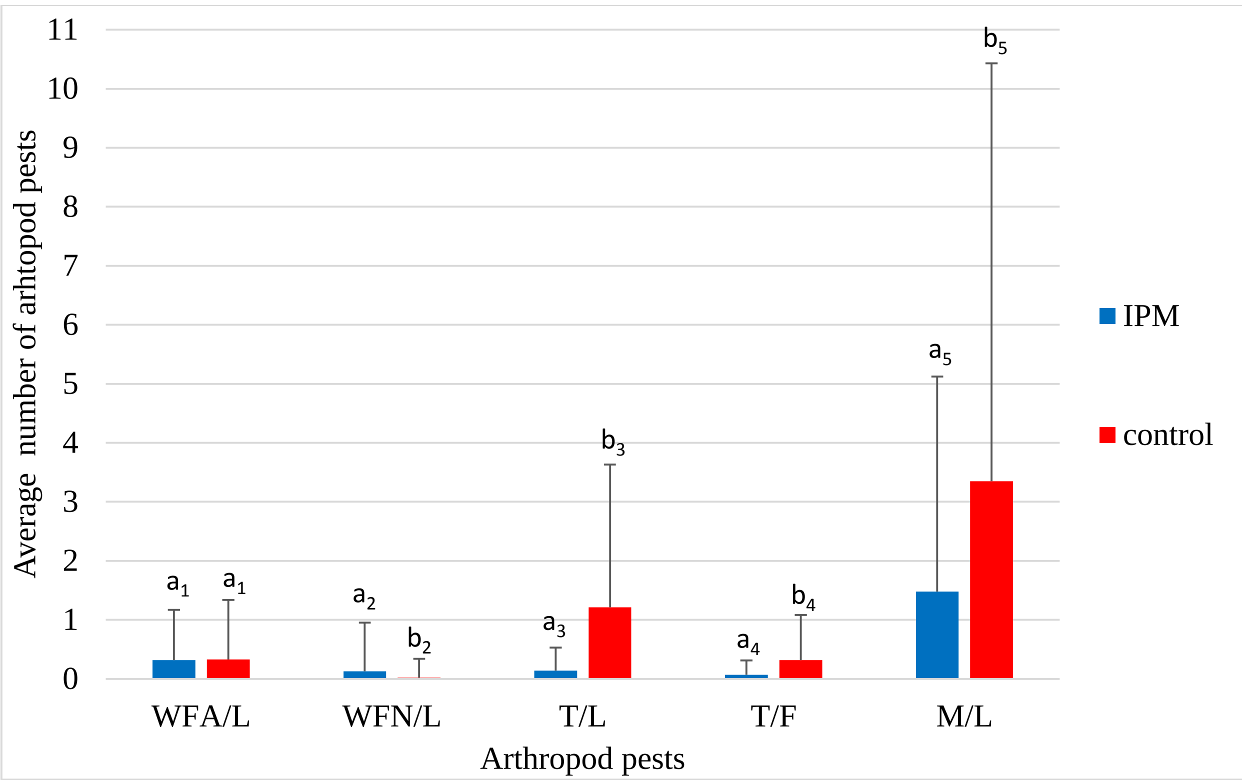


Figure 8. Averages of insects/mite populations in the IPM greenhouse compared to those in the control greenhouse, at site A. Different letters indicate statistically significant difference ($P<0.05$). WFA/L: Whitefly adult per Leaf; WFN/L: Whitefly nymph per Leaf; T/L: Thrips/Leaf; T/F: Thrips/Flower; M/L: Spider-mites/Leaf

The effects of time, pest control program, and pest control program*time on arthropod pest populations were significant ($P = 0.000$), except for that of whitefly adults ($P > 0.05$). In both greenhouses, the averages of whitefly adult and nymph populations were below the ETL. As for thrips and mite, population averages in the IPM greenhouse were maintained below the ETL; 0.14 ± 0.39 thrips/leaf, 0.07 ± 0.24 thrips/flower, and 1.48 ± 3.64 mites/leaf while in the control greenhouse, population averages exceeded the ETL; 1.21 ± 2.42 thrips/ leaf, 0.32 ± 0.76 thrips/ flower, and 3.35 ± 7.08 mites/ leaf. These results ascertain the efficacy of the biologically based IPM approach in suppressing arthropod pest infestations.

The results of the statistical analysis conducted to compare the efficiency of the IPM strategy to that of the conventional plant protection strategy, in terms of all arthropod pests, are presented in Figure 9.

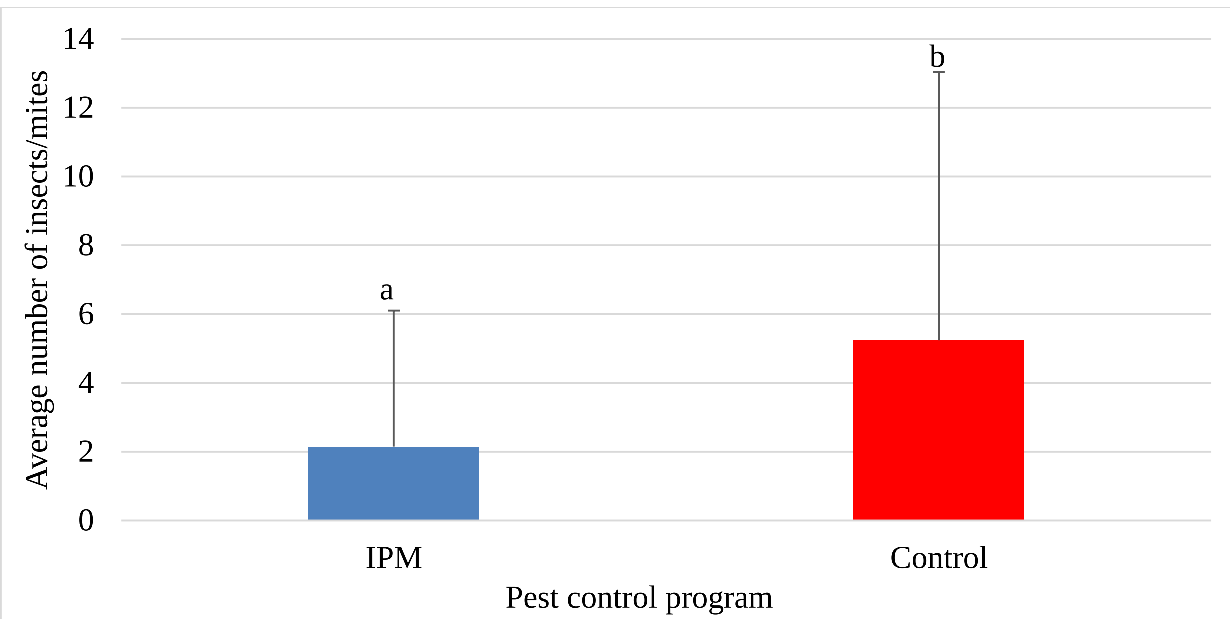


Figure 9. Average population of insects/mite in the IPM greenhouse compared to that in control greenhouse, at site A. Different letters indicate statistically significant difference ($P<0.05$). IPM: Integrated pest management strategy; Control: Conventional pest control strategy

The results showed that the average insects/mite population, recorded in IPM greenhouse, was 2.15 ± 3.95 insects/mites per leaf and flower and was significantly lower (P=0.000) than that in control greenhouse; 5.24 ± 7.80 insects/mites per leaf and flower. Thus, biologically based IPM approach was more successful in reducing total insects/mite population in the greenhouse (by around 60%) than the conventional plant protection strategy. Moreover, it allowed a 63% reduction in total insecticidal/acaricidal applications as compared to the control greenhouse.

b. Site B

The population dynamics of whiteflies, thrips, and mites and their corresponding natural enemies, in the control and IPM greenhouses, are presented in Figures 10-15.

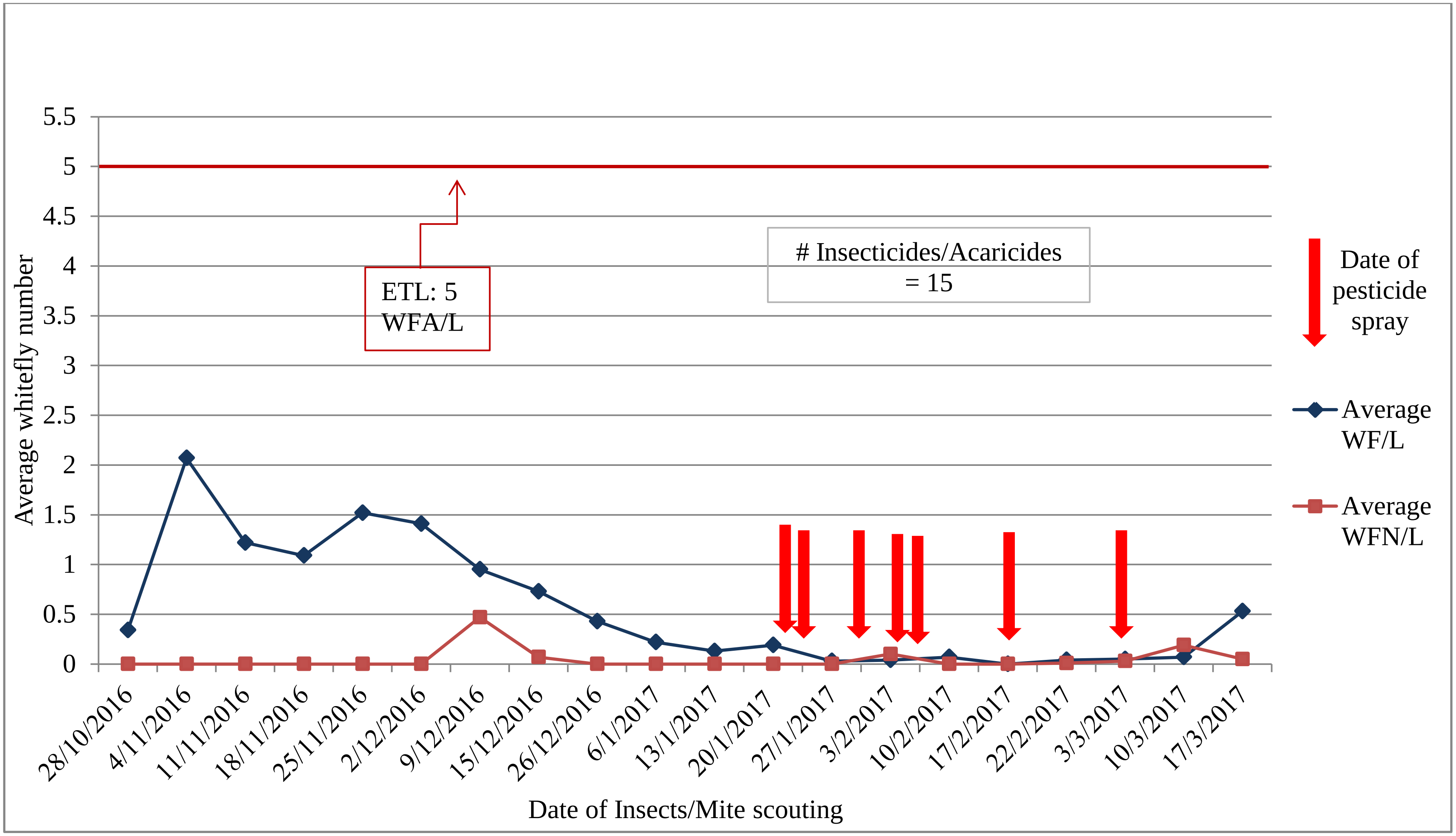


Figure 10. Average whitefly adult and nymph populations recorded on cucumber leaves throughout the growing period in the control greenhouse at site B. A total of 15 insecticidal/acaricidal sprays were performed in the greenhouse. WF/L: Whitefly adult per leaf; WFN/L: Whitefly nymph per leaf; ETL: Economic threshold level

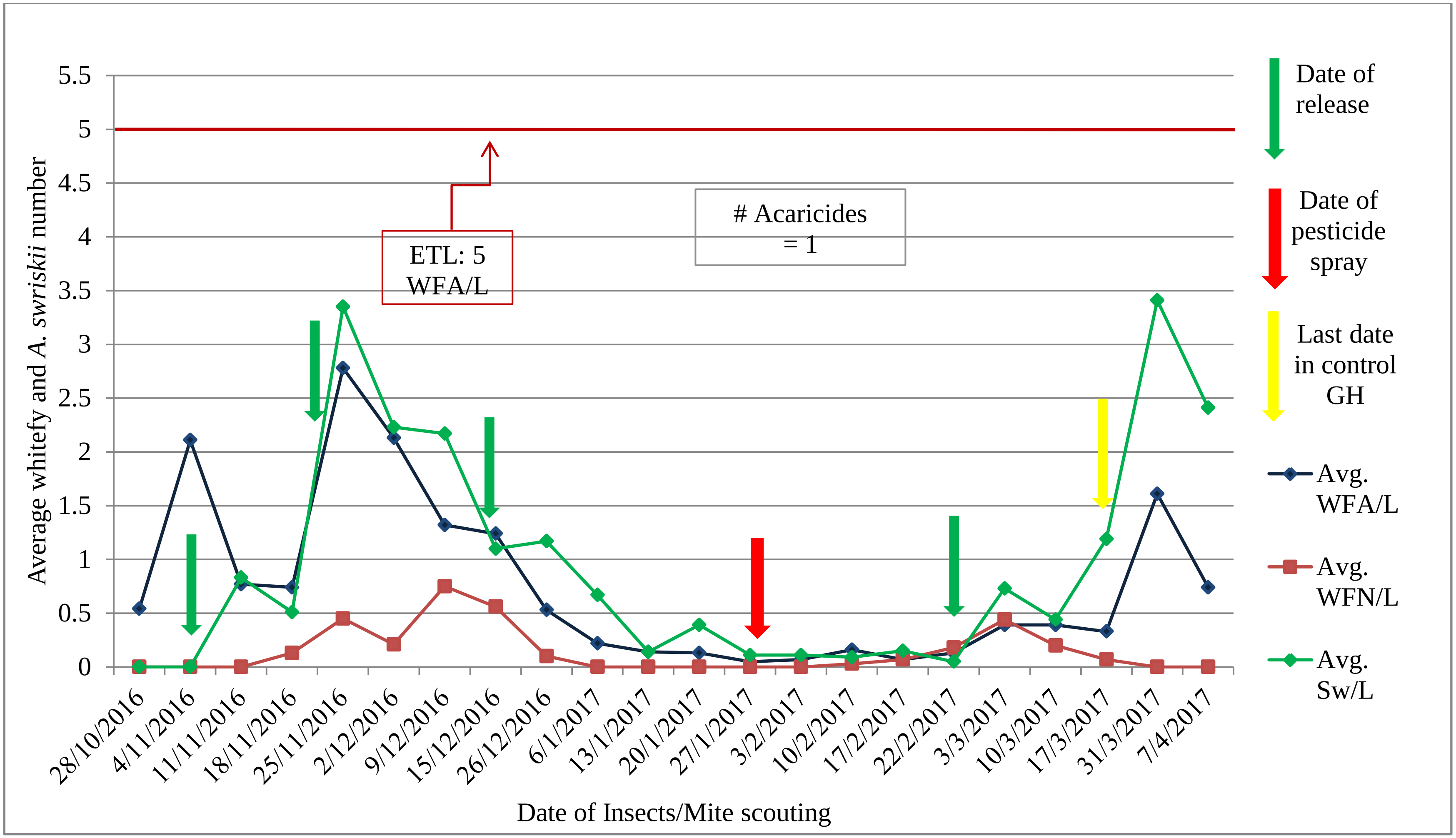


Figure 11. Average whitefly nymph and adult and *Amblyseius swirskii* populations recorded on cucumber leaves throughout the growing period in the IPM greenhouse, at site B. A total of 4 *A. swirskii* introductions and 1 acaricidal application were performed in the greenhouse. WF/L: Whitefly adult per leaf; WFN/L: Whitefly nymph per leaf; Sw/L: *Amblyseius swirskii* per leaf; ETL: Economic threshold level

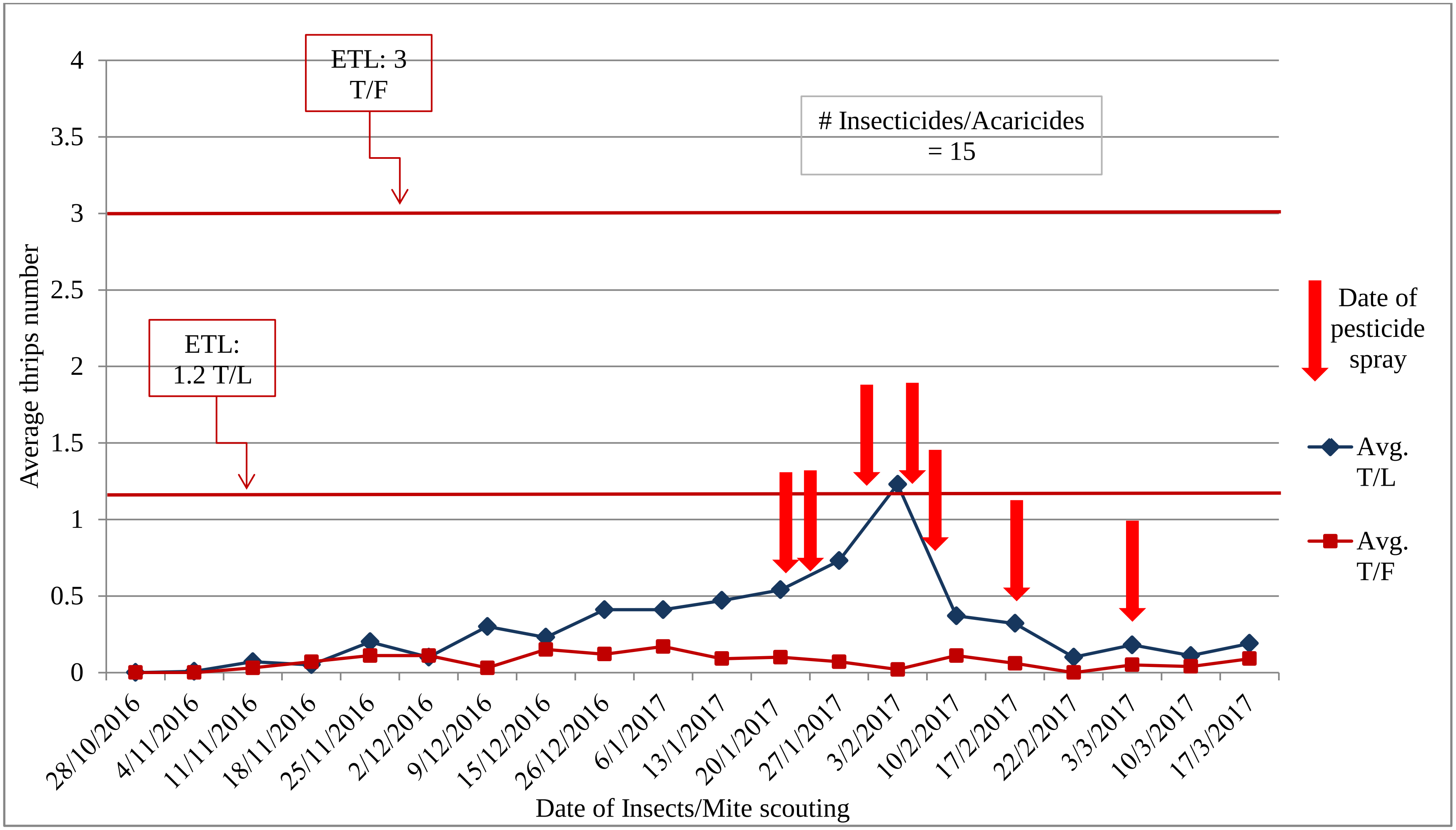


Figure 12. Average thrips population recorded on cucumber leaves and flowers throughout the growing period in the control greenhouse, at site B. A total of 15 insecticidal/acaricidal applications were performed in the greenhouse. T/L: Thrips per leaf; T/F: Thrips per flower; ETL: Economic threshold level

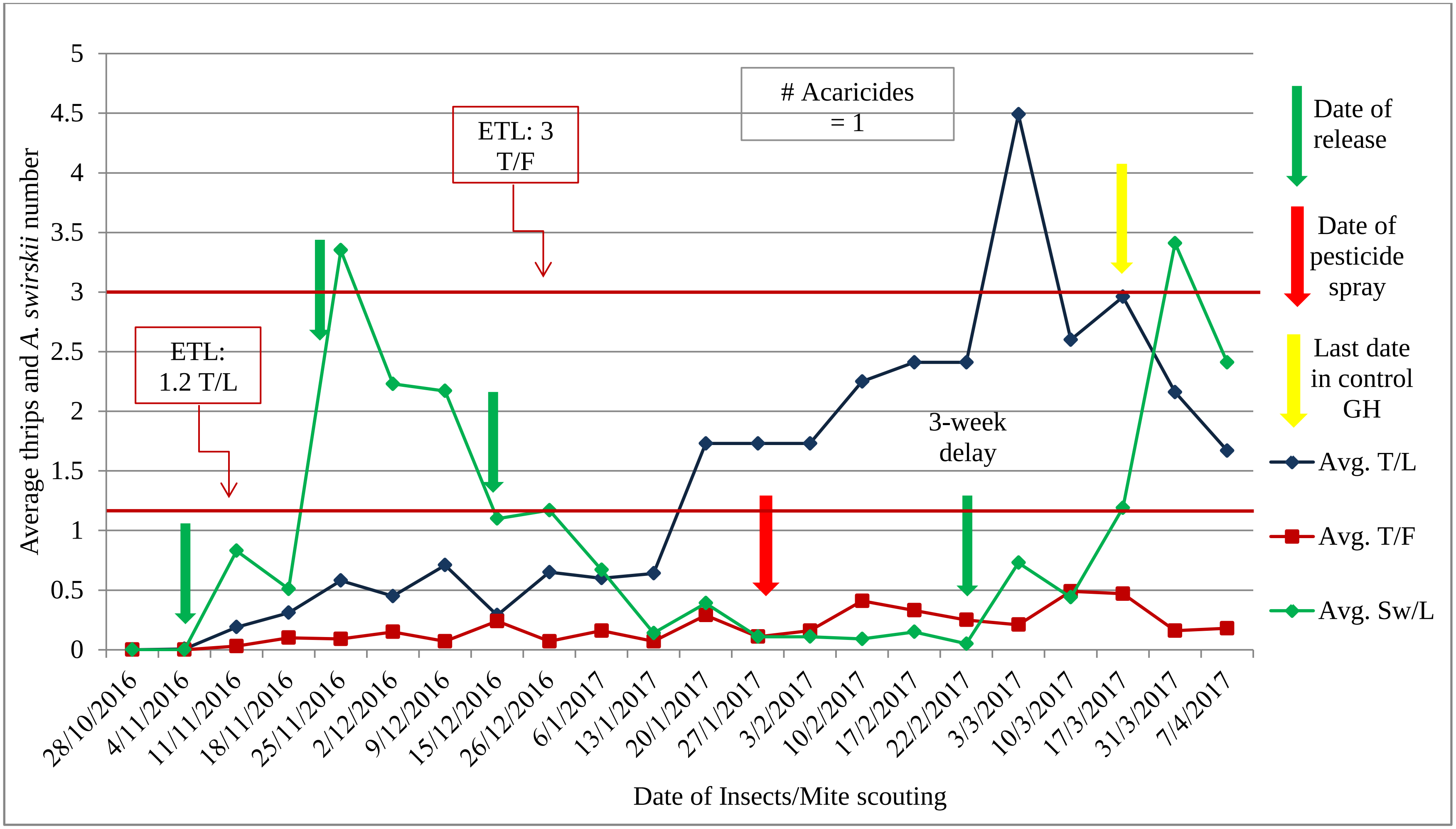


Figure 13. Average thrips populations recorded on cucumber leaves and flowers and *Amblyseius swirskii* population recorded on cucumber leaves in the IPM greenhouse, at site B. A total of 4 *A. swirskii* introductions and 1 acaricidal application were performed in the greenhouse. T/L: Thrips per leaf; T/F: Thrips per flower; Sw/L: *Amblyseius swirskii* per leaf; ETL: Economic threshold level

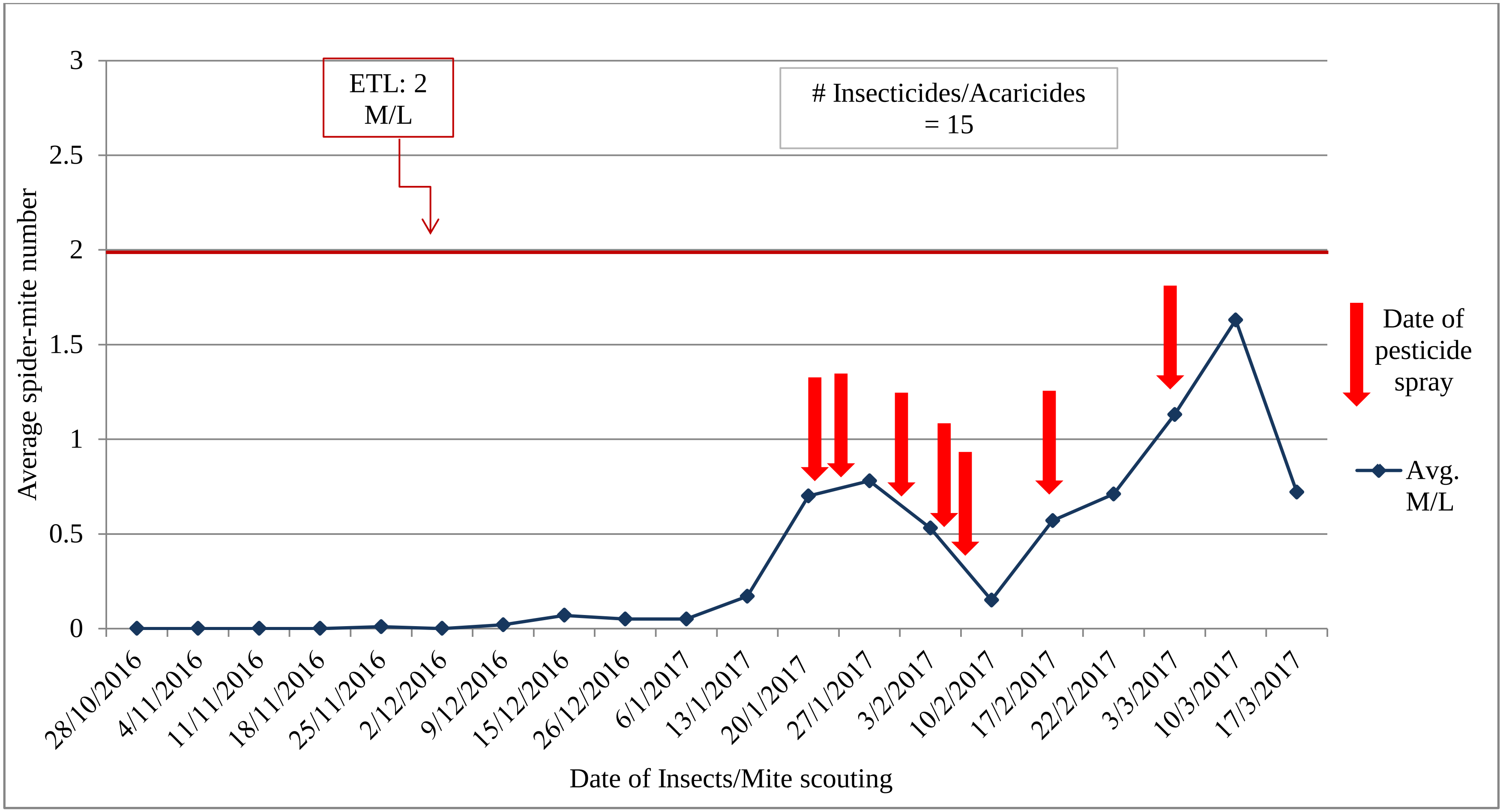


Figure 14. Average spider-mite population recorded on cucumber leaves throughout the growing period in the control greenhouse, at site B. A total of 15 insecticidal/acaricidal sprays were performed in the greenhouse. M/L: Spider-mites per leaf; ETL: Economic threshold level

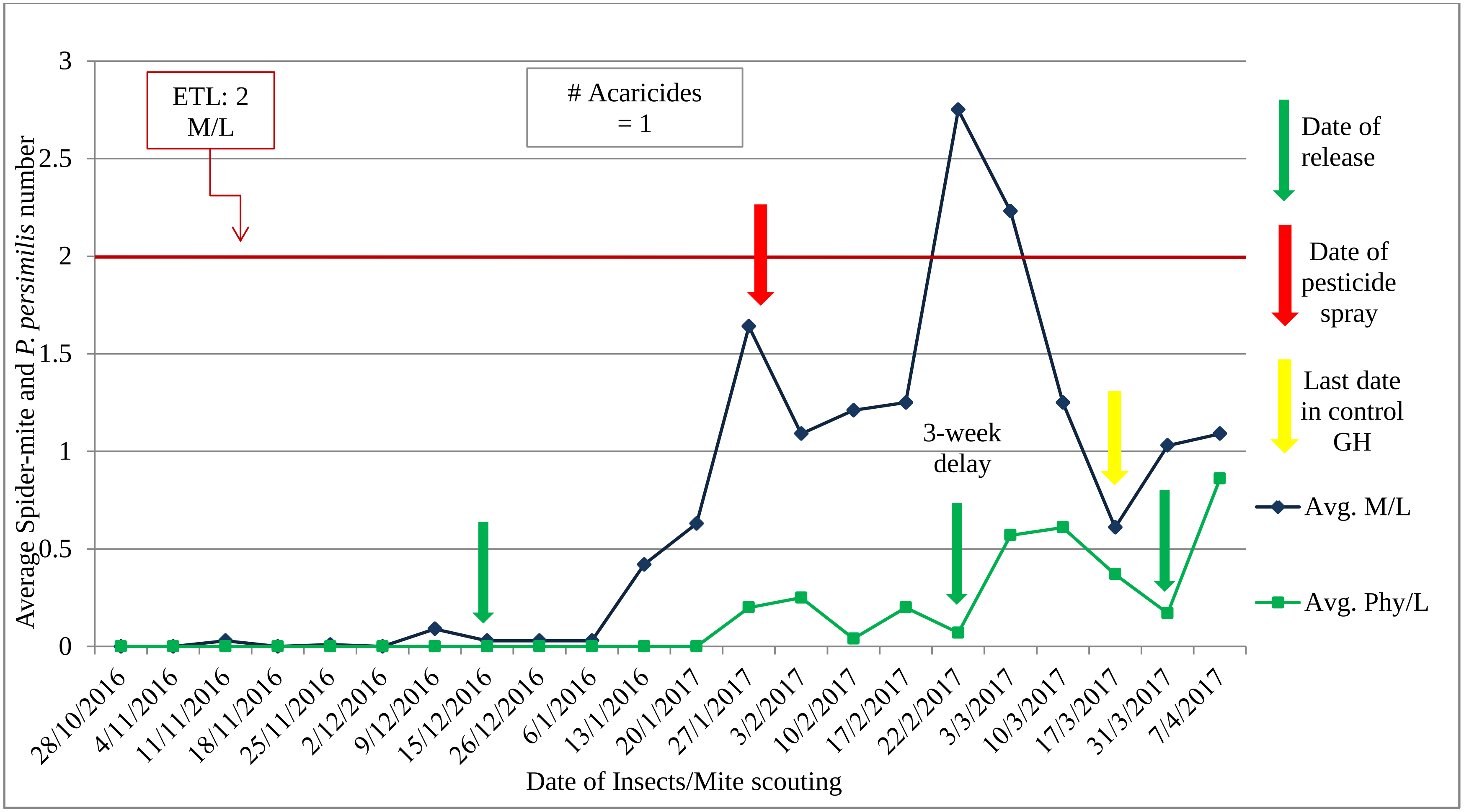


Figure 15. Average spider-mites and *Phytoseiulus persimilis* populations recorded on cucumber leaves throughout the growing period in the IPM greenhouse, at site B. A total of 3 *P. persimilis* introductions and 1 acaricidal application were performed in the greenhouse. M/L: Spider-mites per leaf; Phy/L: *Phytoseiulus persimilis* per leaf; ETL: Economic threshold level

In the control greenhouse, the farmer relied heavily on insecticides/acaricides to control the arthropod pests. Whitefly adult and nymph populations were maintained below the ETL throughout the growing period, with the highest peaks of 2.1 adults and 0.5 nymphs per leaf recorded in early November and in the 2nd week of December, respectively. The high number of whitefly adults reported at the beginning of the season was mainly due to insect migration into the greenhouse since whitefly nymph population was negligible (Fig. 10). Similarly, thrips population on cucumber leaves and flowers were maintained most of the time below 0.5 insect/leaf or flower. However, during the period from mid-January till early February, thrips population on leaves increased from 0.5 to 1.23 insects per leaf (Fig. 12). The mite population was also maintained at below a level of 2 mites/leaf throughout the season, with the highest population reaching over 1 mite/leaf only during the first two weeks of March (Fig. 14). Based on these results, the farmer was able to maintain all arthropod pests at or below their respective ETL, but at high environmental and economic costs due to the frequent pesticide applications that totaled around 15 sprays, each consisting of a mixture of 3 to 5 pesticides.

In the IPM greenhouse, five natural enemy releases and one acaricide application were conducted to suppress arthropod pest population. During most of the season, *A. swirskii* population was well established in the greenhouse, with the highest peaks of 3.35 and 3.41 predator/ leaf recorded in mid-November and end of March, respectively. Due to the high activity of *A. swirskii*, whitefly nymph population in the greenhouse was maintained below 0.5 nymphs/ leaf. Considerable whitefly adult migrations into the greenhouse were encountered at the beginning and at the end of the season, where peaks of 2.78 and 1.61 insect adults were reported. However, its population didn't exceed the ETL

(Fig. 11). These results prove the high efficacy of *A. swirskii* for the management of whiteflies. In a study conducted by Calvo et al. (2011), *A. swirskii,* released at a rate of 75 predators/m^2, led to a successful suppression of whiteflies on cucumber plants. In many other studies, the high activity of *A. swirskii* against whiteflies on cucumber plants was also reported (Stansly & Natwick, 2009). As for thrips population, the first three releases of *A. swirskii* maintained the insect population below 0.75 thrips/leaf or flower till mid-January. After this period, moderately high fungal disease (mainly downy mildew) incidence was recorded in the greenhouse due to high RH (>90%), which persisted for more than 3 hours a day. These conditions forced the farmer to perform two consecutive fungicidal sprays (azoxystrobin + dimethomorph + fenhexamid and thiophanate-methyl + fosethyl aluminum) in a period of around two weeks. As result, *A. swirskii* population was negatively affected by the moderate toxicity of the applied chemicals (based on pesticide side-effect list provided by BioBest©) and was suppressed to less than 0.2 predators/ leaf. The low predator population along with a 3-week delay in natural enemy introduction which should have taken place early February and suitable environmental conditions, allowed thrips population to increase reaching the highest peak of 4.5 thrips/ leaf during the first week of March (Fig.16). Moreover, this considerable increase in thrips number might also be related to the predator preying preference on whitefly eggs (Buitenhuis et al., 2015; Janssen & Sabelis, 2015; Messelink & Janssen, 2008). The last introduction of *A. swirskii* which was performed on the third week of February reduced the insect population from 4.5 to less than l.7 thrips/leaf within four weeks. This was associated with an increase in the predator population to about 3.5/ leaf (Fig. 13). Similar results were reported by Calvo et al. (2011), whereby a release rate of 75 *A. swirskii/* m^2 has significantly suppressed thrips population on cucumber plants. Kutuk et al. (2011), has also reported the high activity of *A. swirskii* against thrips on sweet pepper plants. The spider mite population remained less than 0.7 mites/leaf till mid-January. After which, it started to increase reaching a maximum number of 2.75 mites/leaf on the third week of February due to the 3-week delay in natural enemy introduction and increase in temperature. The introduction of *P. persimilis* at that time at a rate of 12.5 predators/ m^2 reduced the population to 0.61 mites/leaf within 3 weeks. This was associated with an increase in the number of the predator, which reached a maximum of 0.6 predator/leaf two weeks post introduction (Fig. 15).

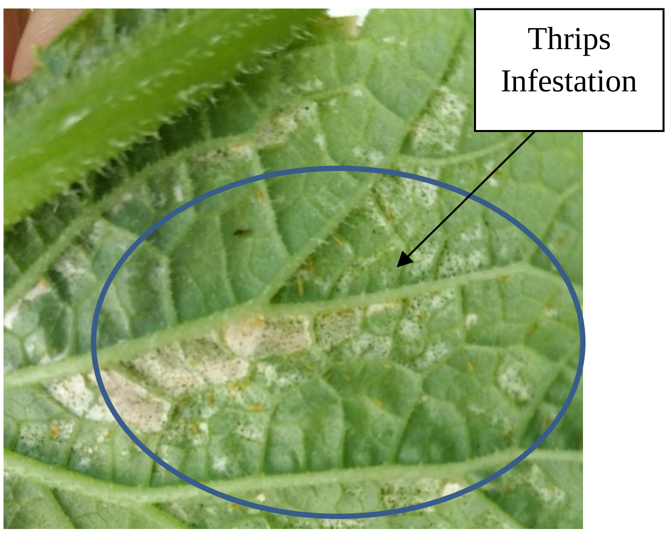


Figure 16. Cucumber plant showing high thrips infestation in the IPM greenhouse, at site B. The silver scares are a result of excessive thrips feeding

Statistically, averages of insects/mite populations were compared using data gathered until the last day of data collection in control greenhouse (17/3/2017). The population averages of whitefly, thrips, and mite in the IPM and control greenhouses are presented in Figure 17.

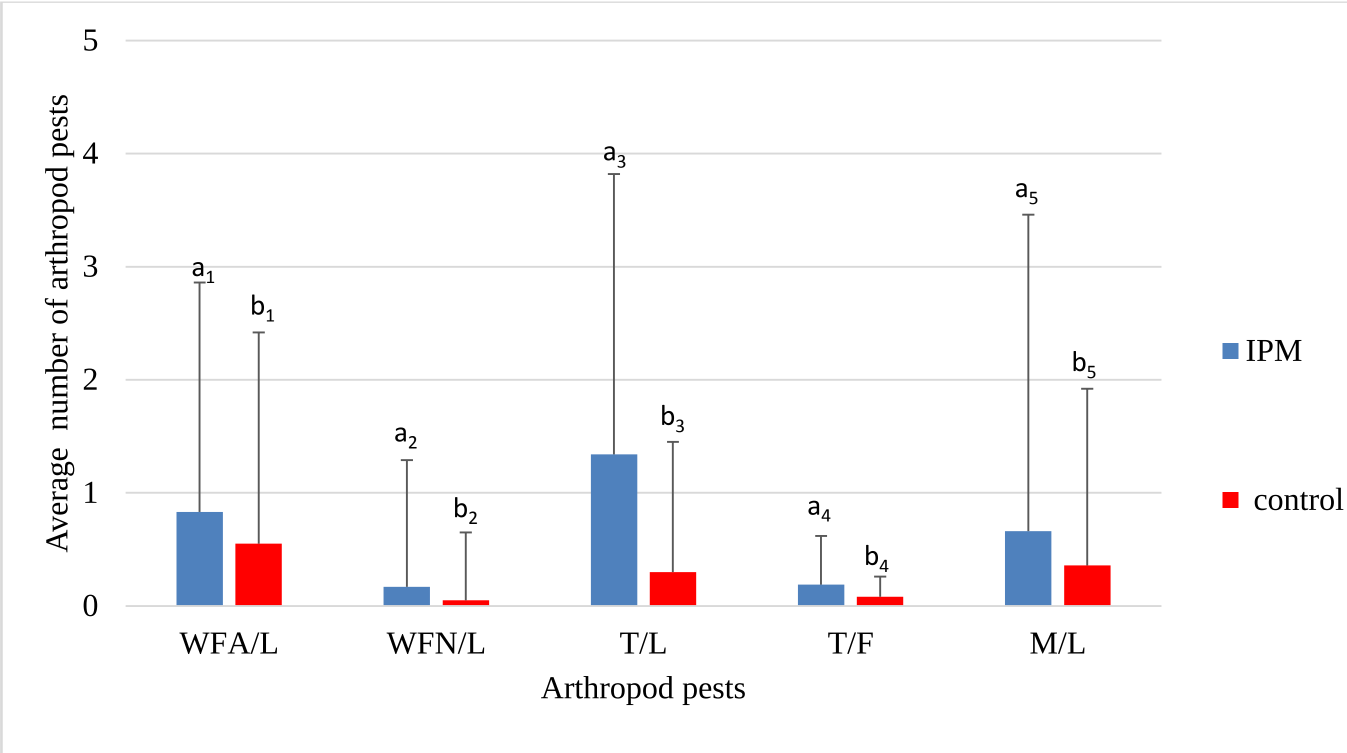


Figure 17. Averages of insects/mite populations in the IPM greenhouse compared to those in the control greenhouse, at site B. Different letters indicate statistically significant difference (P<0.05). WFA/L: Whitefly adult per Leaf; WFN/L: Whitefly nymph per Leaf; T/L: Thrips/Leaf; T/F: Thrips/Flower; M/L: Spider-mites/Leaf

The effects of time, pest control program, and pest control program*time on arthropod pest populations were significant (P = 0.000). Averages of all arthropod pest populations in the IPM greenhouse were higher than those in the control greenhouse however; their populations were maintained below the ETL or 1 insect/mite per leaf, except for thrips which reached 1.34 ± 2.48 insect/leaf in the IPM greenhouse.

The results of the statistical analysis conducted to compare the control efficiency of the IPM strategy to that of the conventional plant protection strategy, in terms of all arthropod pests, are presented in Figure 18.

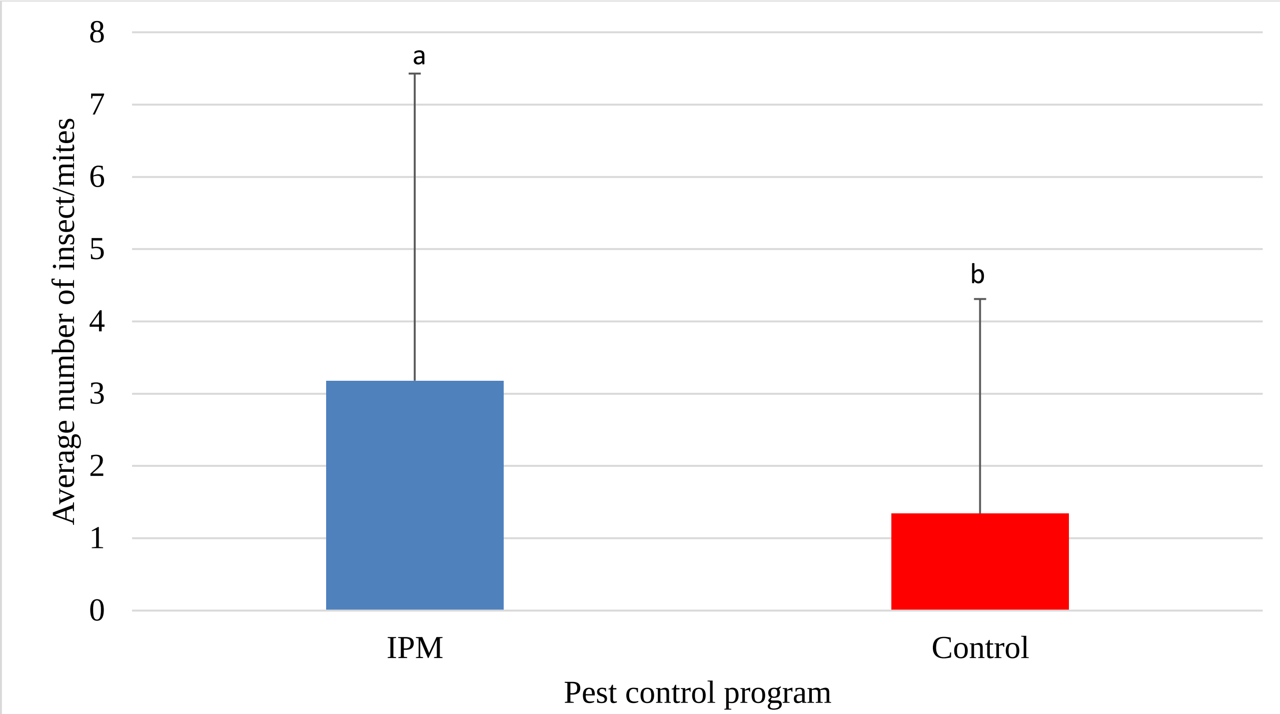


Figure 18. Average insects/mite population in IPM greenhouse compared to that in control greenhouse, at site B. Different letters indicate statistically significant difference ($P<0.05$). IPM: Integrated pest management strategy; Control: conventional pest control strategy

The results showed that the average insects/mite population in IPM greenhouse (3.18 ± 4.25 insects/mites per leaf and flower) was significantly higher ($P=0.000$) than that in control greenhouse (1.34 ± 2.97 insects/mites per leaf and flower). Although the heavy applications of insecticides/acaricides yielded better pest suppression than the biologically based IPM approach, the average number of all arthropod pests per leaf/flower in the IPM greenhouse was maintained at an acceptable level with a 93% reduction in pesticide sprays.

3. *Effect of fungicide applications on disease development and severity in the IPM and control greenhouses*

a. Site A

In the control and IPM greenhouses, powdery mildew was the main fungal disease infecting cucumber plants since the selected variety was relatively susceptible to the disease causal agent, *P. fusca*. Although 3 fungicidal sprays (penconazole, krezoxim-methyl + thiophanate-methyl, and krezoxim-methyl + thiophanate-methyl) were performed in both greenhouses on the same dates, the disease severity index reached around 5% and 15% in the IPM and control greenhouses, respectively. This variation in disease severity was mainly due to the IPM practices followed in the IPM greenhouse, which included the removal of highly infected leaves and plants. As for downy mildew disease, its severity was minimal and somewhat similar in both greenhouses, taking into consideration that only one fungicide targeting downy mildew (propamocarb-HCl) was sprayed throughout the growing period. This shows that the environmental conditions were not optimal for downy mildew disease progression, specifically the maximum RH which was around 80% during the growing period (Savory et al., 2011).

b. Site B

Throughout the experimental period, high RH and low aeration were recorded in the greenhouses. These conditions were suitable for fungal disease infections, including gray mold (*Botrytis*), sclerotinia, and powdery mildew. In addition, downy mildew disease outbreak was encountered in both greenhouses since the cucumber variety was quite susceptible to the disease causal agent, *P. cubensis.* Although frequent fungicide applications (a mixture of at least 2 fungicides per spray) were performed in the control greenhouse at roughly weekly interval, the disease severity index of downy mildew exceeded 35% (i.e. leaf area infected) by the end of the growing period. While in the IPM greenhouse, it reached around 50% despite of three selective fungicidal sprays. Moreover, considerable damage caused by the other listed fungi was also reported on cucumber plants in both greenhouses, forcing the farmer to remove the crop. These observations show that in humid environments, an integration of several control practices including selection of downy mildew tolerant/resistant cucumber variety, proper aeration and selective fungicidal applications is essential to successfully manage fungal disease infections (Savory et al., 2011).

4. ***Effect of fungicide applications on A. swirskii and P. persimilis populations, in the IPM greenhouses***

a. Site A

The effect of fungicides on the predatory mites could not be determined since their populations were very low before and after fungicidal sprays. However, the applied pesticides are listed by BioBest© as relatively not toxic to the released natural enemies, except for thiophanate-methyl which is reported as toxic to *P. persimilis.* The latter fungicide was only applied when *P. persimilis* population was not observed in the greenhouse.

b. Site B

The application of azoxystrobin + dimethomorph + fenhexamid and myclobutanil didn’t seem to have a negative impact on *P. persimilis* since a continuous increase in the predator population was recorded one week after fungicide applications. In contrast, a decrease in its number was reported after the application of thiophanate-methyl and fosethyl aluminum, which shows that they might be slightly toxic to the predator. These observations are in agreement with the pesticide side-effect list provided by BioBest©, whereby thiophanate methyl is considered a toxic fungicide to *P. persimilis* while the other fungicides mentioned above are relatively harmless. On the other hand, myclobutanil might have had a slight side effect on *A. swirskii* population since a decrease in its number was observed post fungicide application. However, rapid recovery in its population has been evident during a period of two weeks, reaching 3.41 *A. swirskii*/leaf. Similarly, the other fungicidal sprays (azoxystrobin + dimethomorph + fenhexamid and thiophanate-methyl + fosethyl aluminum) were somewhat toxic to the latter predator since its population was suppressed for almost one to two weeks after pesticide application, knowing that all the other factors (prey availability, temperature, and RH) that might affect *A. swirskii* development were suitable.

5. ***Cucumber yield produced from the IPM and control greenhouses and at both locations***

At Site A, the total cucumber yield produced in the IPM greenhouse was 912 Kg (0.96 Kg/plant) while that produced in the control greenhouse was 504 Kg (0.53 Kg/plant). The difference in the crop yield was mainly due to higher arthropod pest infestation in the control greenhouse compared to that in the IPM greenhouse. As for the low production quantity per plant in both greenhouses, it is hypothesized to be due to high concentration of accumulated salts in the coco-peat substrate since it was used for two consecutive seasons. At site B, the total cucumber yield produced in the IPM greenhouse was 5157 Kg (5.73 Kg/plant) while in the control greenhouse, it reached 5904 Kg (6.56 Kg/plant). The difference in crop yield was mainly due to higher fungal disease incidence in the IPM greenhouse compared to that in the control greenhouse. The yield data prove that the IPM program can maintain high crop-yield potential and at instances produces higher production quantity when compared to the conventional pest control program and this mainly achieved by reducing arthropod pest infestation in the greenhouse.

6. *Comparison between yellow sticky cards (YSCs) and cucumber leaves in attracting whitefly adults, in IPM greenhouses*

The averages of whitefly adult populations attracted to YSCs and cucumber leaves, at both locations, are presented in Figure 19.

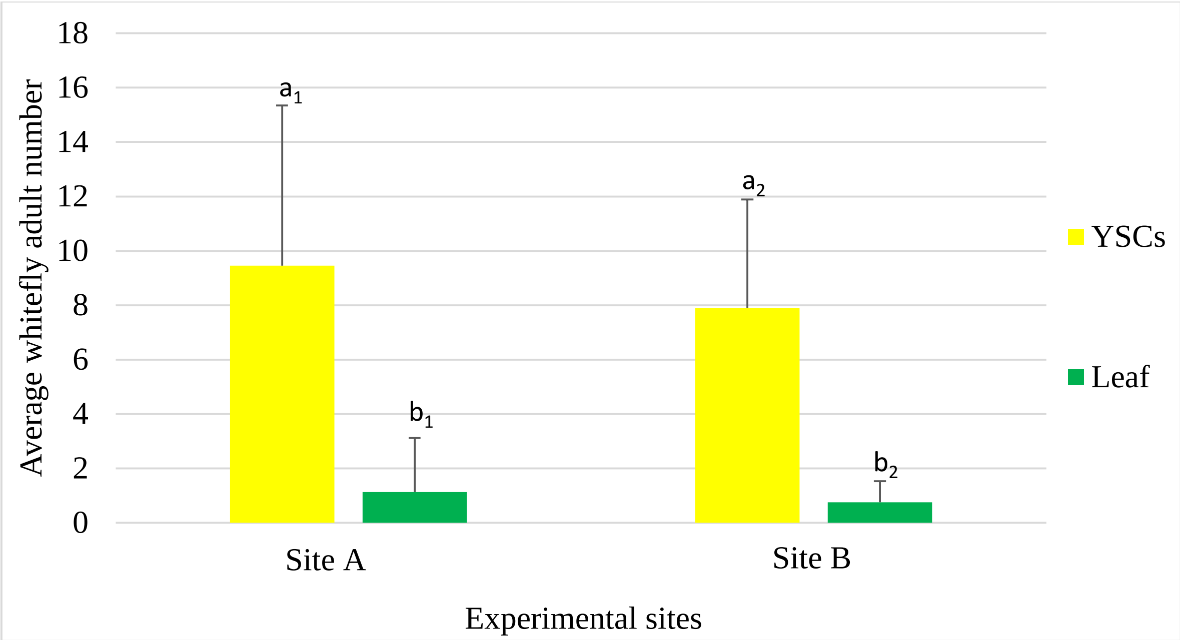


Figure 19. Averages of whitefly adult populations trapped on yellow sticky cards (YSCs) and cucumber leaves in the IPM greenhouses, at sites A and B. Different letters indicate statistically significant differences ($P < 0.05$). YSCs: Yellow sticky cards

At sites A and B, averages of whitefly adult populations trapped on YSCs were respectively 9.45 ± 5.9 and 7.89 ± 4.00 insects/YSCs/week and were significantly higher (P_1=0.000; P_2=0.000) than those attracted to cucumber leaves; 1.13 ± 1.99 and 0.75 ± 0.78 whitefly adults/ leaf. This shows that YSCs can be used as an effective tool to monitor and reduce whitefly population in cucumber-grown greenhouses. Moreover, the considerable difference between average whitefly adults trapped on YSCs and average whitefly nymphs recorded on cucumber leaves at site A (0.39 nymphs/leaf) and site B (0.14 nymphs/leaf), proves that insect adult migrations, from near vegetative area into the greenhouses, have occurred.

7. ***Comparison between blue sticky cards (BSCs) and cucumber leaves/flowers in attracting thrips, in IPM greenhouses***

The averages of thrips populations trapped on BSCs and cucumber leaves at both locations are presented in figure 20.

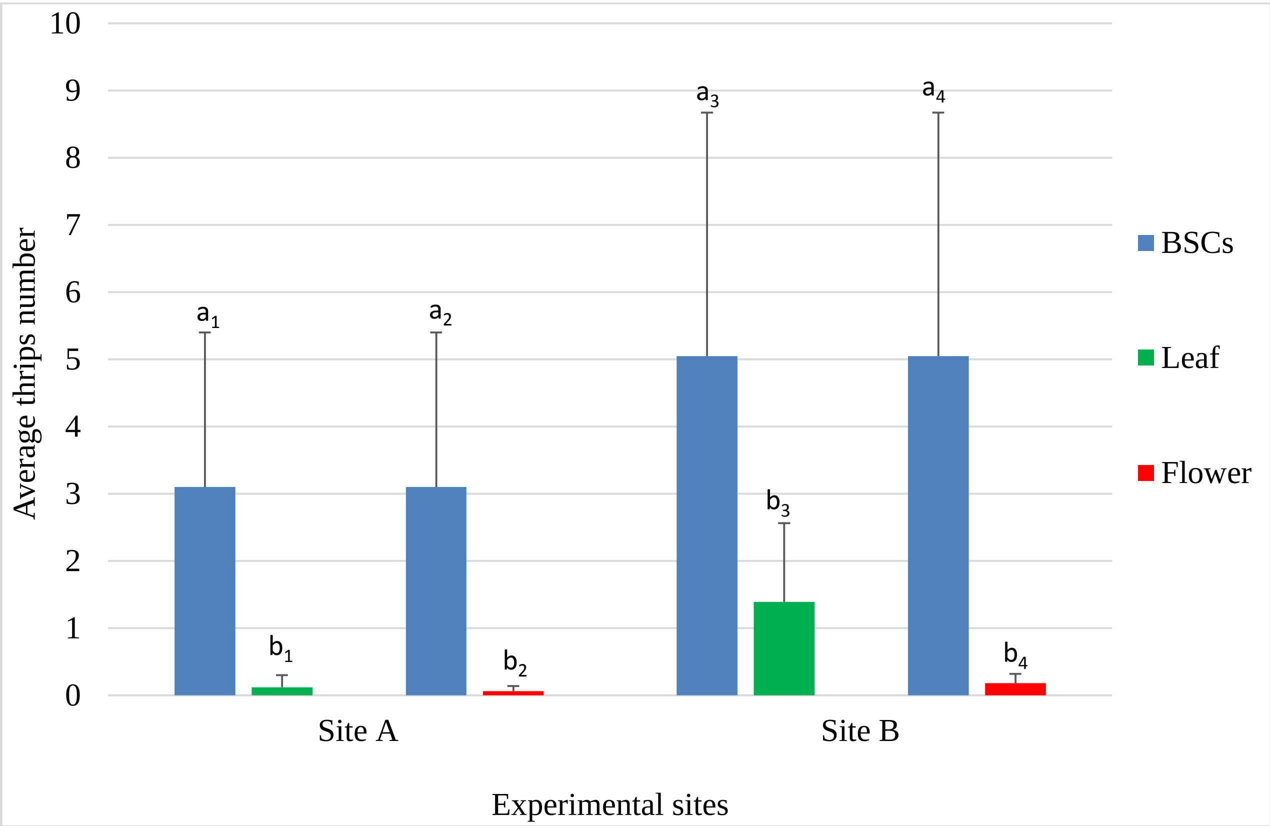


Figure 20. The averages of thrips populations trapped on blue sticky cards (BSCs) and cucumber leaves/flowers in the IPM greenhouses, at sites A and B. Different letters indicate statistically significant differences ($P < 0.05$). BSCs: Blue sticky cards

At site A, average thrips population trapped on BSCs (3.10 ± 2.30 thrips/BSCs/week) was significantly higher (P_1=0.000; P_2=0.000) than that attracted to cucumber leaves; 0.12 ± 0.18 adult/leaf, and cucumber flowers; 0.06 ± 0.08 adult/flower, respectively. Similarly, at site B, significant differences (P_1=0.000; P_2=0.000) were recorded between average thrips number caught on BSCs (5.05 ± 3.62 thrips/BSCs/week) and that recorded on cucumber leaves (1.39 ± 1.17 insect/leaf) and flowers (0.18 ± 0.14 insect/flower), respectively. Based on these observations, the BSCs could be used in cucumber-grown greenhouses as a monitoring tool for thrips population. However, the high thrips numbers on BSCs compared to cucumber leaves and flowers, at both locations, might also be associated to the predation activity of *A. swirskii*.

8. *Comparison between marigold plants and cucumber leaves/flowers in attracting thrips, in IPM greenhouses*

The averages of thrips populations trapped on potted marigolds and cucumber leaves/flowers are presented in Figure 21. The average of thrips populations trapped on ground marigolds and cucumber leaves/flowers, at site B, are presented in figure 22.

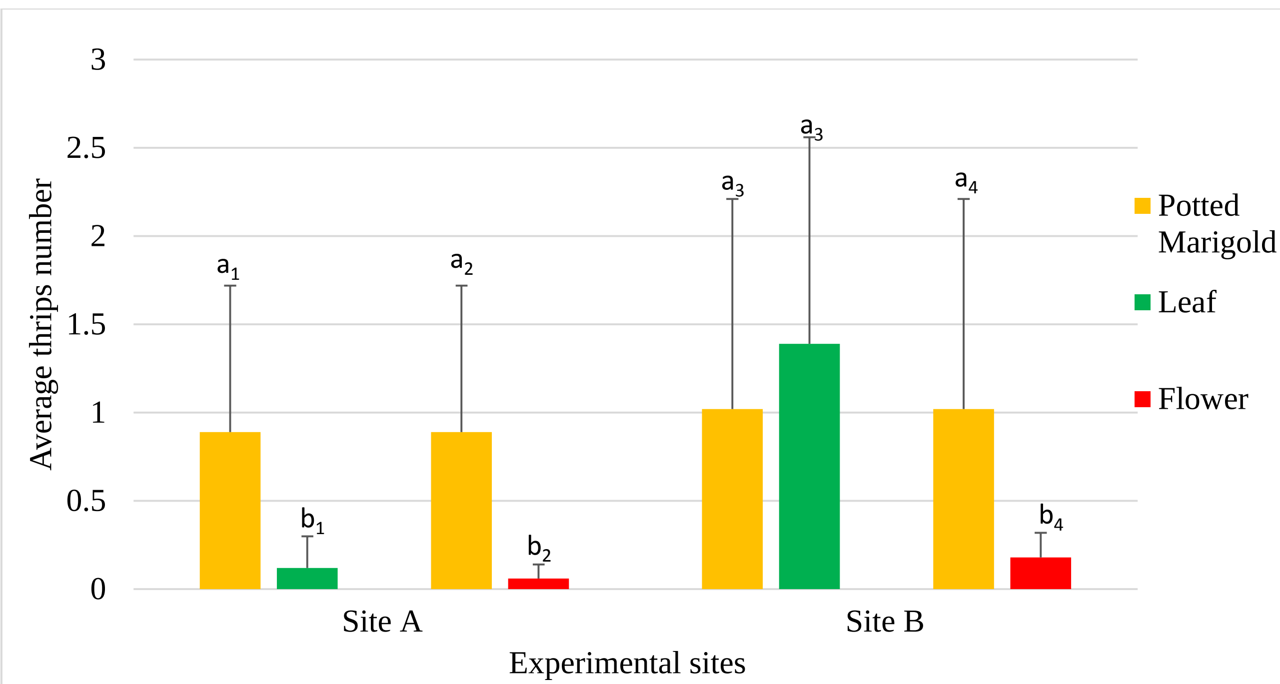


Figure 21. The averages of thrips populations trapped on potted marigolds and cucumber leaves/flowers, in the IPM greenhouses, at both locations

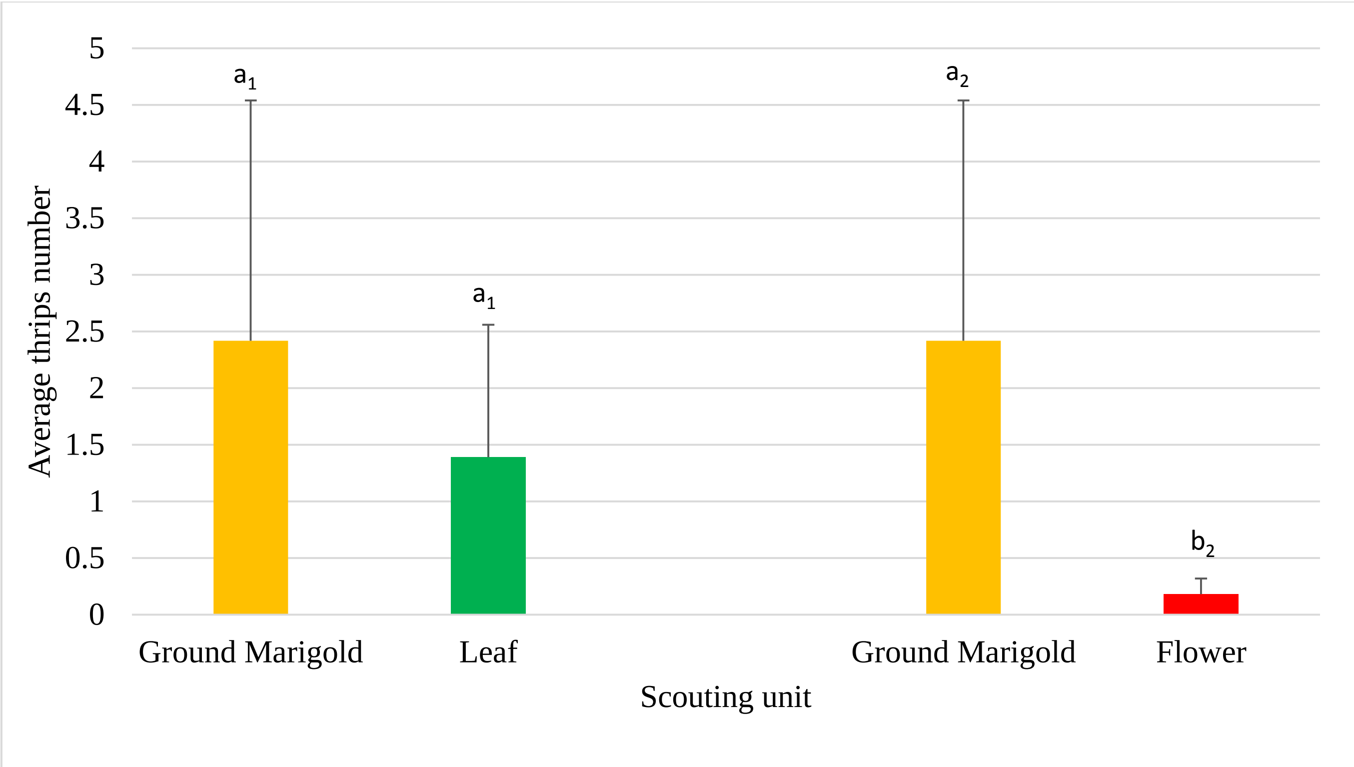


Figure 22. The averages of thrips populations trapped on ground marigolds and cucumber leaves/flowers in the IPM greenhouse, at site B. Different letters indicate statistically significant differences ($P < 0.05$)

At Site A, the average of thrips population attracted to potted marigolds was 0.89 ± 0.83 thrips/flower and was significantly higher (P_1=0.000; P_2=0.000) than that attracted to cucumber flowers (0.06 ± 0.08 thrips/flower) and cucumber leaves (0.12 ± 0.18 thrips/leaf). At site B, significant result (P=0.002) was recorded when comparing average thrips population attracted to potted marigolds (1.02 ± 1.19 thrips/ flower) and that recorded on cucumber flowers (0.18 ± 0.14 thrips/flower) while, no significant difference (P=0.304) was observed between average thrips number on marigolds and cucumber leaves (1.39 ± 1.17 thrips/ leaf).

At site B, the average of thrips trapped on ground marigolds was 2.42 ± 2.12 thrips/flower and was significantly higher (P=0.000) than that attracted to cucumber flowers (0.18 ± 0.14 adult/flower), but not significantly different (P=0.053) than that attracted to cucumber leaves (1.39 ± 1.17 adult/ leaf).

These results show that marigold flowers transplanted in pots were less attractive to thrips than marigolds planted in the ground soil since potted flowers were poorly irrigated and not well fertilized. Moreover, marigolds may not act as a good banker/habitat plant and/or monitoring tool for thrips infestation in cucumber-grown greenhouses.

B. Evaluation of the efficacy of *Beauveria pseudobassiana* against *Tuta absoluta*, under laboratory conditions

The effect of the applied treatments on *T. absoluta* eggs was clear 6-7 days post application. Infected eggs (non-hatched) turned, progressively, from milky-white to dark brown in color and subsequently were covered with white mycelial growth (Fig. 23). Some of the infected eggs had successfully hatched; however, the emerged larva was not able to burrow tunnels more than 5 mm in length and/or was shriveled in appearance and dark brown in color (Fig. 24). On the other hand, healthy eggs did not show any sign of infection and had successfully hatched after an incubation of 4-5 days and the emerged larva was able to survive and burrow a tunnel exceeding 5 mm in length.

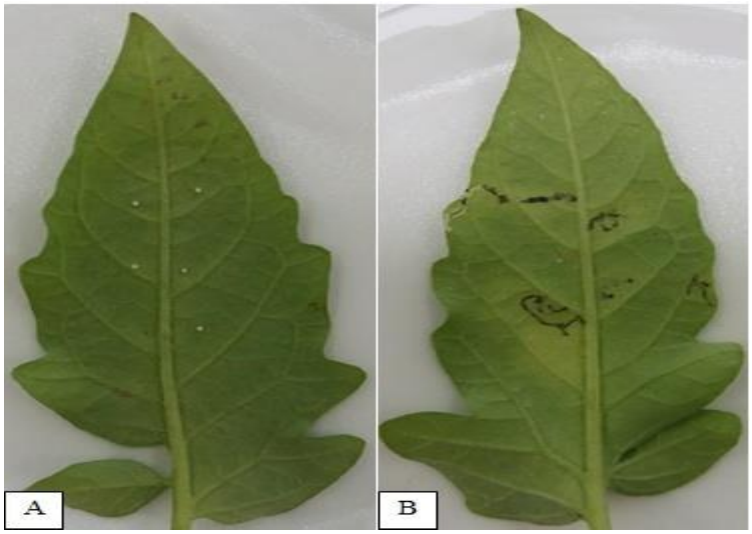


Figure 23. Detached tomato leaves with *Tuta absoluta* eggs. A: Eggs sprayed with 10^8 conidia mL^{-1} + 0.01% Tween-20 + 1% corn oil showing fungal sporulation 7 days post-inoculation; B: Eggs sprayed with 0.01% Tween-20 + 1% corn oil showing larvae mines. Both leaves were incubated at the same time and under same conditions

Figure 24. *Tuta absoluta* larvae at the same developmental stage. On the left, healthy larva from negative control while on the right, both larvae are infected with *Beauveria pseudobassiana*

1. *Preliminary insect bio-assay*

A total of 390 eggs were used in this preliminary bioassay to evaluate the efficacy of *B. pseudobassiana* on the egg stage of *T. absoluta*. The mean percent mortalities are presented in table 6.

Table 6. Preliminary bioassay: Efficacy of the *Beauveria pseudobassiana* conidial suspensions, with and without adjuvants, against *T. absoluta* eggs, 7 days post-inoculation. Mean percent mortality (%) of six replicates, each consisting of five eggs, is calculated for each treatment

Treatment #	**Treatment**	**Corrected mortality (%) ***
1	Water control	0[a]
2	Tween	6.67[ab]
3	Tween + Corn oil	10[abc]
4	Tween + Codacide® oil	3.33[a]
5	10^6 conidia mL^{-1} + Tween	38.33[cd]
6	10^6 conidia mL^{-1} + Tween +Corn oil	47.5[cde]
7	10^6 conidia mL^{-1} + Tween + Codacide® oil	26.67[abc]
8	10^7 conidia mL^{-1} + Tween	50[cde]
9	10^7 conidia mL^{-1} + Tween +Corn oil	73.33[e]
10	10^7 conidia mL^{-1} + Tween + Codacide® oil	33.33[bc]
11	10^8 conidia mL^{-1}+ Tween	82.5[e]
12	10^8 conidia mL^{-1} + Tween +Corn oil	92.5[e]
13	10^8 conidia mL^{-1} + Tween + Codacide® oil	64.17[de]
	Total number of eggs	390

* Mean mortality with different letters indicate statistically significant differences (LSD test, $P < 0.05$)

The percentage mortalities recorded in the four negative controls were not significantly different from one another and varied between 0, 3.33, 6.67 and 10% for treatments 1, 4, 2, and 3, respectively. These results show that the adjuvants used had only a minor effect on egg mortality. The highest percentage mortalities of 82.5% and 92.5% were recorded on eggs sprayed with the highest conidial concentration, 10^8 conidia mL^{-1}, without or with 1% corn oil, respectively. The latter adjuvant has enhanced control efficacy of *Beauveria* in treatments involving lower conidial concentrations (47.5% and 73.33% for treatments 6 and 9, respectively). Similar observation was reported in other trials whereby, corn oil added to the spray mixture has slightly increased the efficacy of control (Abou-Jawdah et al., 2008), mainly by improving the adherence of *Beauveria* spores to *T. absoluta* eggs or larvae or to minor toxic effect that weakens the insect and renders it more susceptible (Camargo et al., 2012). On the other hand, Codacide® affected the results negatively by reducing mean percent mortalities (26.67, 33.33, and 64.17% for treatments 7, 10, and 13, respectively).

The linear regression analysis was significant (P=0.000) with an R-squared of 0.9211 and proved that fungal efficacy against *T. absoluta* is dependent on conidial concentration. At 10^6conidia mL^{-1}, the mortalities were 48% - 50% and at 10^7 conidia mL^{-1}, it ranged between 73% - 84.3% while, at the highest conidial concentration it ranged between 92.5% and 100% (Fig. 25).

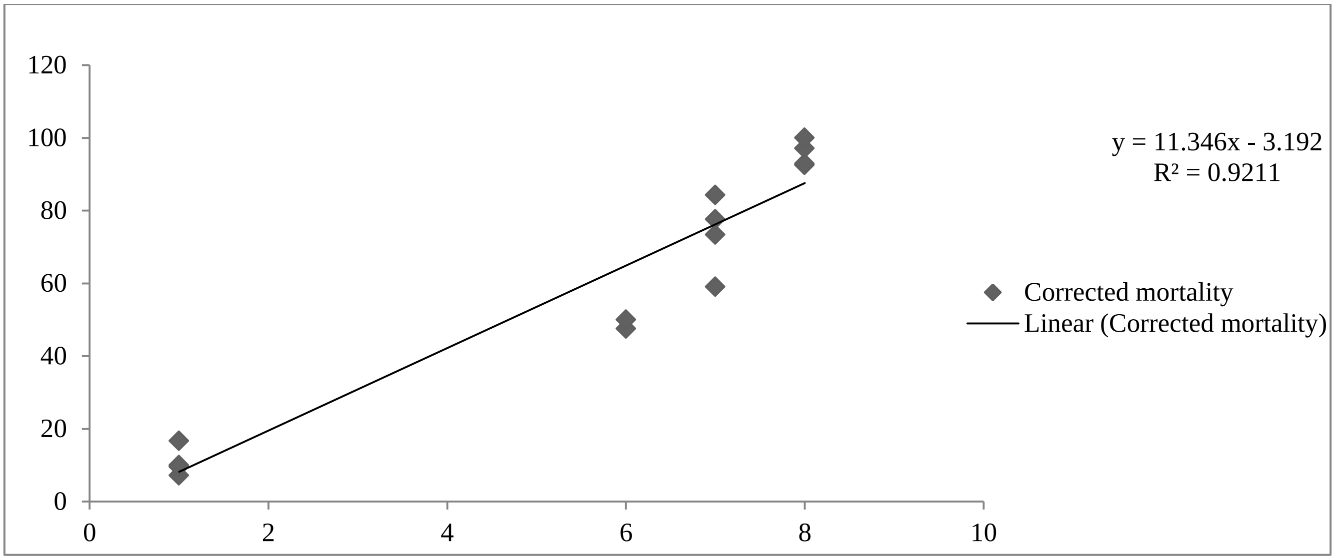


Figure 25. Log *Beauveria* conidial concentrations with respect to percent mortalities of *T. absoluta* eggs

2. *In –vitro bio-assays; egg and larval susceptibility*

A total of 756 eggs were used in the three performed bioassays. The mean percent mortality of each treatment is presented in table 7. Statistical analysis was conducted for the trials separately followed by split plot analysis for the means of the 3 bioassays.

Based on the results of the preliminary bioassay and regression analysis, treatments including 0.01% Tween-20 not amended with any of the two adjuvants, 0.01% Tween-20 + 0.1% Codacide®, and 10^6 conidia/ mL + 0.01% Tween-20 + 1% corn oil, were eliminated due to their low efficacy, in terms of egg mortality, compared to the other treatments. The average mortality for each treatment is presented in Table 7.

Table 7. Efficacy of control of *T. absoluta* by *B. pseudobassiana* as compared to abamectin and *B. thuringiensis*. Results of each bioassay were the averages of seven replicates each consisting of six eggs (42 eggs/treatment)

	Bioassay 1			**Bioassay 2**			**Bioassay 3**			**Overall (Split-Plot analysis)**
Treatments	**Not hatched/ Sporulated Eggs**	**Dead Larvae**	**Average Corrected Mortality (%)***	**Not Hatched/ Sporulated Eggs**	**Dead Larvae**	**Average Corrected Mortality (%)**	**Not hatched/ Sporulated Eggs**	**Dead Larvae**	**Average Corrected Mortality (%)**	**Average Corrected Mortality (%)**
Water only	0%	0%	0^{a**}	0%	2.4%	0^{a}	0%	2.4%	0^{a}	0^{a}
Water + Tween-20 + Corn oil	0%	7.1%	7.14^{a}	0%	7.9%	16.7^{a}	0%	7.3%	9.52^{a}	11.11^{b}
10^{7} conidia mL^{-1} + Tween-20 + Corn oil	61.9%	23.8%	84.3^{c}	56.4%	7.7%	59^{b}	43.2%	35.1%	77.62^{c}	73.65^{c}
10^{8} conidia mL^{-1} + Tween-20 + Corn oil	92.8%	0%	92.86^{cd}	91.7%	5.5%	97.14^{c}	95.2%	4.8%	100^{d}	96.67^{d}
B. thuringiensis	0%	60.9%	61.9^{b}	0%	71.8%	72.86^{b}	0%	63.1%	62.85^{b}	65.87^{c}
Abamectin	100%	0%	100^{d}	100%	0%	100^{c}	100%	0%	100^{d}	100^{d}

* Average total mortality= egg mortality + larval mortality
**Averages with different letters indicate statistically significant differences (LSD test, $P < 0.05$)

The effects of the applied treatments on egg mortality were significant in all three replicated bioassays (P_1=0.000; P_2=0.000; P_3=0.000). The results of the statistical analysis, conducted for each of the 3 bioassays separately, confirmed those obtained in the preliminary trial. The *B. pseudobassiana* treatment, consisting of the highest conidial concentration (10^8 conidia mL^{-1}), resulted in high mortalities ranging between 92.86% and 100%, which were numerically and significantly higher than that involving lower conidial concentration (10^7 conidia mL^{-1}), except in the first trial where no significant difference was recorded between the two treatments. Even though abamectin gave the highest mortality rate of 100%, this mortality was not significantly different from that of the *Beauveria* treatment consisting of the highest conidial concentration. *B. thuringiensis*; the most common biological control agent against *T. absoluta*, was effective only on larvae and gave mortalities ranging from 61.9% to 72.86%. These mortalities were significantly lower than those observed in *Beauveria* treatments (10^7 and 10^8 conidia mL^{-1} with adjuvant), except in the second trial where *B. thuringiensis* treatment resulted in numerically higher mortality compared to treatment with 10^7 conidia mL^{-1}. As for the two negative controls, their average mortalities were not significantly different from one another (Table 7). However, when a Split plot analysis of the means for the three bioassays was performed, the results showed that the percent mortality of 0% in the water control and 11.11% in water + 0.01% Tween-20 + 1% corn oil, were significantly different from one another and both were significantly different from the other treatments. It seems that the 1% corn oil alone had minor toxic effect on the eggs (Table 7). Moreover, no significant difference was recorded between *B. pseudobassiana* treatment with 10^7 conidia mL^{-1} and *B. thuringiensis*.

This in–vitro study showed that the local isolate of *B. pseudobassiana*, was quite effective in the control of *T. absoluta*. Spraying 10^8 conidia mL^{-1}+ 0.01% Tween-20 + 1% corn oil at the egg stage resulted in a significant mortality of eggs as well as of neonate larvae with a total mortality ranging between 92% and 100%. Our data are in strong agreement with other researchers who showed that *B. bassiana* can infect *T. absoluta* eggs (Pires et al., 2009; Shalaby et al., 2013).

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

In Lebanon, the most important greenhouse-grown crops are tomato and cucumber. Greenhouse environmental conditions allow higher plant yield and fruit quality. At the same time, it favors an increase in arthropod pest populations and disease incidences. Most of the famers apply mixtures of toxic pesticides, on a weekly basis, to overcome the latter agricultural challenges. Consequently, several regions have encountered a rapid build-up of insect resistance, along with severe health and environmental problems. Nowadays, most farmers in developed countries adopt biologically-based IPM strategies to suppress arthropod pest populations. Multiple success stories related to the high efficacy of the natural enemies, including *A. swirskii* and *P. persimilis,* were reported in many European countries and some states in USA (Van-Lenteren et al., 2018). Alongside the mentioned beneficial organisms, the entomopathogenic fungi *B. bassiana* and *M. anisopliae* have proven to be efficient control agents of several insect pests including *T. absoluta* (Inanli et al., 2012).

The laboratory study focused on the application of *B. pseudobassiana* as an alternative control measure of *T. absoluta,* through the artifical infection of 1,146 insect eggs. Successfully infected eggs turned dark-brown in color and were consequently covered with white mycelial growth. While infected larvae were shriveled in appearance, brownish in color, and were unable to burrow tunnels of more than 5 mm in length. The results of this in-vitro study showed that *B. pseudobassiana* was highly effective in controlling the egg and larval stages of *T. absoluta,* in a dose-dependant infection model. The activity of the fungal entomopathogen was improved with the addition of 1% corn oil to the conidial suspensions. In contrast, the adjuvant codacide® acted as an antagonist by reducing the activity of *B. pseudobassiana*. The highest mortalities of 92.5% to 100% were recorded on eggs treated with 10^8 conidia mL^{-1} + 0.01% Tween 20 + 1% corn oil while, the lowest mortality (<51%) was associated with the lowest conidial suspension of 10^6 conidia ml^{-1} + 0.01% Tween 20 + 1% corn oil. The efficacy of *B. pseudobassiana*, applied at the highest concentration, was not significantly different from that of the reference insecticide/acaricide, abamectin ®. However, it was more effective than the reference microbial organism, *B. thurengiensis*, which gave a mortality of less than 73% in the three conducted bioassays.

The conducted field studies at two environmentally different locations, aimed at assessing the efficacy of *A. swirskii*, *P. persimilis*, and *E. eremicus* in reducing whitefly, thrips and mite populations to below damaging levels with minimal applications of toxic insecticides/acaricides. The pre-transplanting IPM measures performed in the IPM greenhouses and the application of broad-spectrum insecticides in the control greenhouses have successfully reduced the initial arthropod pest populations. During the experimental period, four natural enemy releases at site A and five at site B aimed at the management of insect/mite populations in IPM greenhouses. When deemed necessary, selective insecticides/acaricides and fungicides were applied to assist the natural enemies in reducing high arthropod pest populations and lower disease incidence. In the control greenhouses, the farmers followed their normal plant protection practices by mainly relying on pesticide sprays.

Throughout the growing period, the temperature and RH recorded at site B (Zahrani) were higher than those at site A (Mount-Lebanon) by around 5°C and 7%, respectively. In the control greenhouse at site A, the farmer was environmentally aware and applied pesticides based on pest monitoring. A total of 8 insecticidal/acaricidal sprays were performed in the greenhouse starting from early January since before that period, arthropod pest population was maintained very low due to relatively low recorded temperature. Throughout the experimental period, the population of whiteflies was suppressed to below the ETL. On the other hand, thrips and mite populations increased rapidly reaching a peak of 2.99 thrips and 20.1 mites per leaf, forcing the farmer to remove the crop. This significant increase in pest populations was mainly due to suitable environmental conditions, resistance of arthropod pests to the applied insecticides/acaricides, and/or inappropriate application of pesticides. In contrast, at site B, the farmer was able to maintain all arthropod pest populations below the ETL during the growing season. This was mainly achieved by 15 applications of insecticide/acaricide mixtures, each consisting of a minimum of 3 pesticides.

In the IPM greenhouses, the population of whitefly nymphs was maintained below the ETL throughout the growing period (mostly less than 1 nymph/leaf), despite of considerable whitefly adult migrations recorded at the beginning and end of the season. These results proved the high prey activity of *A. swirskii* on the egg and nymphal stages of whiteflies, knowing that a single female whitefly adult can lay up to 200 eggs during its life span on cucumber plants. *A. swirskii* was also highly effective in maintaining thrips population on cucumber leaves and flowers to below damaging levels in the IPM greenhouse at site A. Similarly, at site B, thrips population on flowers did not exceed 0.5

insect/flowers, during the growing period. As for thrips number on cucumber leaves, it has increased to above the EIL, during the period from mid January till early March. This is explained by the low *A. swirskii* population recorded during that period (negatively affected by two consecutive fungicidal sprays); a 3 weeks delay in natural enemy introduction, preying preference of the predatory mite on whitefly eggs and nymphs, and an increase in temperature which favored thrips development. However, upon the introduction of the predator (3rd week of February), thrips population was reduced to 1.7 insect/leaf, within less than four weeks. Concerning the mite population, it was maintained at very low level (less than 0.25 mites/leaf) during November and December at both locations. However, starting from mid-January till the 3rd week of February at site B and from mid-February till early April at site A, the mite population increased rapidly reaching a peak of 2.75 and 8.09 mites/leaf, respectively. These observations were due to a 3-weeks delay in the 2nd introduction of the natural enemy, low *P. persimilis* populations, and an increase in average and maximum temperatures. Moreover, at site A, the 2nd introduction of the predator (3rd week of February) was not effective due to a 24-hours delay in release after receipt along with unfavorable storage conditions which have adversly affected the quality of the predator. However, upon the last effective introduction of *P. persimilis* at both locations, the mite population dropped, within 3-4 weeks, to more than 50% following an increase in the number of the predator. This shows the high efficacy of *P. persimilis* in controlling spider mite infestation, when applied at the proper time and rate. As for the parsitic wasp, *E. eremicus*, its quality was very low due to unfavorable storage conditions during transportation to Lebanon and thus we were unable to assessits efficacy in controlling whiteflies.

In conclusion, this study showed a high potential for the use of predators and parasites to manage arthropod pest infestations under normal greenhouse conditions. Based on the results of the in-vitro study, *B. pseudobassiana*, applied at a concentration of 10^8 conidia mL^{-1} + 1% corn oil + 0.01% tween-20, is very effective in controlling *T. absoluta*. Moreover, the efficacy of the local *Beauveria* isolate is higher than that of the most commonly applied microbial agent, *Bacillus thuringiensis*, and similar to that of the reference insecticide, abamectin. As for the field studies, the results proved that the two predatory mites, *A. swirskii* and *P. persimilis*, are highly effective in suppressing whitefly, thrips, and mite populations to below ETL and can allow 63%-93% reduction in insecticidal/acaricidal applications. Noting that, for a successful insect pest control, the natural enemies must be released at the appropriate time and rate, should be of high quality, and assisted by selective pesticides to control plant diseases and reduce pest infestations without adversely affecting their populations.

<u>Recommendations:</u>

For an effective biologically-based IPM in Lebanon, the following recommendations must be taken into consideration:

i. Conduct field experiments to test the efficacy of *B. pseudobassiana* under field conditions.

ii. Rearing natural enemies locally to minimize transportation costs, maintain high quality of the beneficial organisms, and prevent delay in greenhouse introductions

iii. Test the efficacy of the locally reared beneficial organisms under greenhouse conditions

iv. Application of selective pesticides to reduce disease infection and high pest infestation without adversely affecting predator populations.

v. Recommend to the Ministry of agriculture to initiate a fast track for registration of selective, safe pesticides.

vi. Selection of resistant/tolerant plant varieties to major fungal diseases to reduce the side-effect of frequent fungicide applications and to maintain crop vigor

vii. Test the potential of other banker/habitat plants to improve the establishment rate of *A. swirskii*

viii. Release *A. swirskii* on banker/habitat plants before the occurrence of whiteflies and thrips in the greenhouse to promote the build-up of the predator population- a preventative measure.

ix. Use high-quality data loggers to record the temperature and RH during the growing season without any malfunctioning

x. Improve greenhouse infrastructure prior to transplanting the crop to reduce arthropod pest migration into the greenhouse

The preliminary results of the biologically based IPM are very encouraging and it is highly recommended to intensify these studies for the safety of people, wild life and the environment, and to allow the farmers to export their produce to the European Union or any other country.

APPENDIX I

PESTICIDE SPRAYS

Table I: List of non-harmful pesticides to natural enemies of interest prepared based on the side effect manual data sets created by Koppert© and Biobest© groups

Natural enemies / Pesticides	*A. swirskii*	*P. persimilis*	*E. eremicus*
Acequinocyl	NT	ST	?
Bifenazate	MD	ST	NT
Cyflumetofen	NT	NT	NT
Spirodiclofen	ST	ST	NT
Propamocarb-HCl	ST	NT	NT
Penconazole	?	NT	NT
Kresoxim methyl	NT	NT	NT
Azoxystrobin	NT	NT	NT
Fenhexamid	NT	ST	?
Myclobutanil	ST	NT	NT
Dimethomorph	MD	NT	?
Fosethyl-aluminum	MD	NT	?
Thiophanate-Methyl	NT	T	NT

Table II: Details of pesticides applied in the experimental greenhouses, at both locations, throughout the growing period of cucumber plants

Farm location	Date of application	Trade name	Active ingredient	Rate of application	Target pest
Site B	28/1/2017	Shine	Azoxystrobin	16mL/20L	Powdery mildew
		Orvego	Dimethomorph	20mL/20L	Downy mildew
		Teldor	Fenhexamid	20mL/20L	Sclerotinia/ botrytis
		Denisaraba	Cyflumetofen	20mL/20L	Mites
	15/2/2017	Actamyl	Thiophanate-methyl	20g/20L	Powdery mildew
		Aliette	Fosethyl-aluminum	70g/20L	Downy mildew
	2/3/2017	Systhane	Myclobutanil	2.4g/20L	Powdery mildew
Site A	8/12/2016	Privent	Propamocarb-HCL	60mL/20L	Downy mildew
	22/12/2016	Topas	Penconazole	10mL/20L	Powdery mildew
	18/1/2017	Kanemite	Acequinocyl	20mL/20L	Mites
	1/2/2017	Actamyl	Thiophanate-methyl	20g/20L	Powdery mildew
		Stroby	Kresoxim-methyl	4g/20L	Powdery mildew
	17/2/2017	Actamyl	Thiophanate-methyl	20g/20L	Powdery mildew
		Stroby	Kresoxim-methyl	4g/20L	Powdery mildew
	10/3/2017	Floramite	Bifenazate	8mL/20L	Mites
	14/3/2017	Envidor	Spirodiclofen	8mL/20L	Mites

Table III: Details of pesticides applied in the control greenhouse throughout the growing period of cucumber plants, at site B.

Farm location	Date of application	Trade name	Active ingredient	Rate of application	Target pest
Site B	10/1/2017	Privent	Propamocarb-HCL	60mL/20L	Downy mildew
		Shine	Azoxystrobin	16mL/20L	Powdery mildew
		Rovral	Iprodione	25g/20L	Botrytis
	21/1/2017	Shine	Azoxystrobin	16mL/20L	Powdery mildew
		Orvego	Dimethomorph	20mL/20L	Downy mildew
		Mospilan	Acetamiprid	10g/20L	Leaf miner
		Diva	Abamectin	10 mL/20L	Mites/whiteflies
		Rovral	Iprodione	25g/20L	Botrytis
	24/1/2017	Shine	Azoxystrobin	16mL/20L	Powdery mildew
		Orvego	Dimethomorph	20mL/20L	Downy mildew
		Diva	Abamectin	10 mL/20L	Mites/whiteflies
		Forest	Pyrimethanyl	30 ml/20L	Botrytis
		Sumi-Alpha	Esfenvalerate	4mL/20L	Thrips
	30/1/2017	Diva	Abamectin	10 mL/20L	Mites/whiteflies
		Mospilan	Acetamiprid	10g/20L	Leaf miner
		Pirate 24 SC	Chlorfenapyr	10mL/20L	Mites/thrips
	4/2/2017	Pirate 24 SC	Chlorfenapyr	10mL/20L	Mites/thrips
		Diva	Abamectin	10mL/20L	Mites/whiteflies
		Forest	Pyrimethanyl	30 ml/20L	Botrytis
		Topas	Penconazole	10mL/20L	Powdery mildew
		Ridomil gold	Metalaxyl +Mancozeb	50/20L	Downy mildew
	7/2/2017	Ridomil gold	Metalaxyl +Mancozeb	50/20L	Downy mildew
		Actamyl	Thiophanate-methyl	20g/20L	Powdery mildew
		Vydate	Oxamyl	200mL/20L	Nematodes
	17/2/2017	Clortosip	Chlorothalonil	76mL/20L	Downy mildew
		Sumi-Alpha	Esfenvalerate	4mL/20L	Thrips
		Actara	Thiamethoxam	10 mL/20L	Whiteflies
		Envidor	Spirodiclofen	8mL/20L	Mites
	3/3/2017	Sumi-Alpha	Esfenvalerate	4mL/20L	Thrips
		Clortosip	Chlorothalonil	76mL/20L	Downy mildew
		Systhane	Myclobutanil	2.4g/20L	Powdery mildew

Table IV: Details of pesticides applied in the control greenhouse throughout the growing period of cucumber plants, at site A

Farm location	Date of application	Trade name	Active ingredient	Rate of application	Target pest
Site A	8/12/2016	Privent	Propamocarb	60mL/20L	Downy mildew
	22/12/2016	Topas	Penconazole	10mL/20L	Powdery mildew
	5/1/2017	Diva	Abamectin	10mL/20L	Mites/whiteflies
	18/1/2017	Kanemite	Acequinocyl	20mL/20L	Mites
	25/1/2017	Kanemite	Acequinocyl	20mL/20L	Mites
	1/2/2017	Actamyl	Thiophanate-methyl	20g/20L	Powdery mildew
		Stroby	Kresoxim-methyl	4g/20L	Powdery mildew
		Marshal	Carbosulfan	30ml/20L	Mites/thrips
	9/2/2017	Pirate 24 SC	Chlorfenapyr	10mL/20L	Mites/thrips
		Nissorun	Hexythiazox	10g/20L	Mites
	14/2/2017	Tracer	Spinosad	3ml/20L	Thrips
	17/2/2017	Actamyl	Thiophanate-methyl	20g/20L	Powdery mildew
		Stroby	Kresoxim-methyl	4g/20L	Powdery mildew
	1/3/2017	Diva	Abamectin	10mL/20L	Mites/whiteflies
	10/3/2017	Floramite	Bifenazate	8mL/20L	Mites

APPENDIX II

TEMPERATURE AND RH

Figure I. Temperature and RH variations recorded throughout the growing period at site A

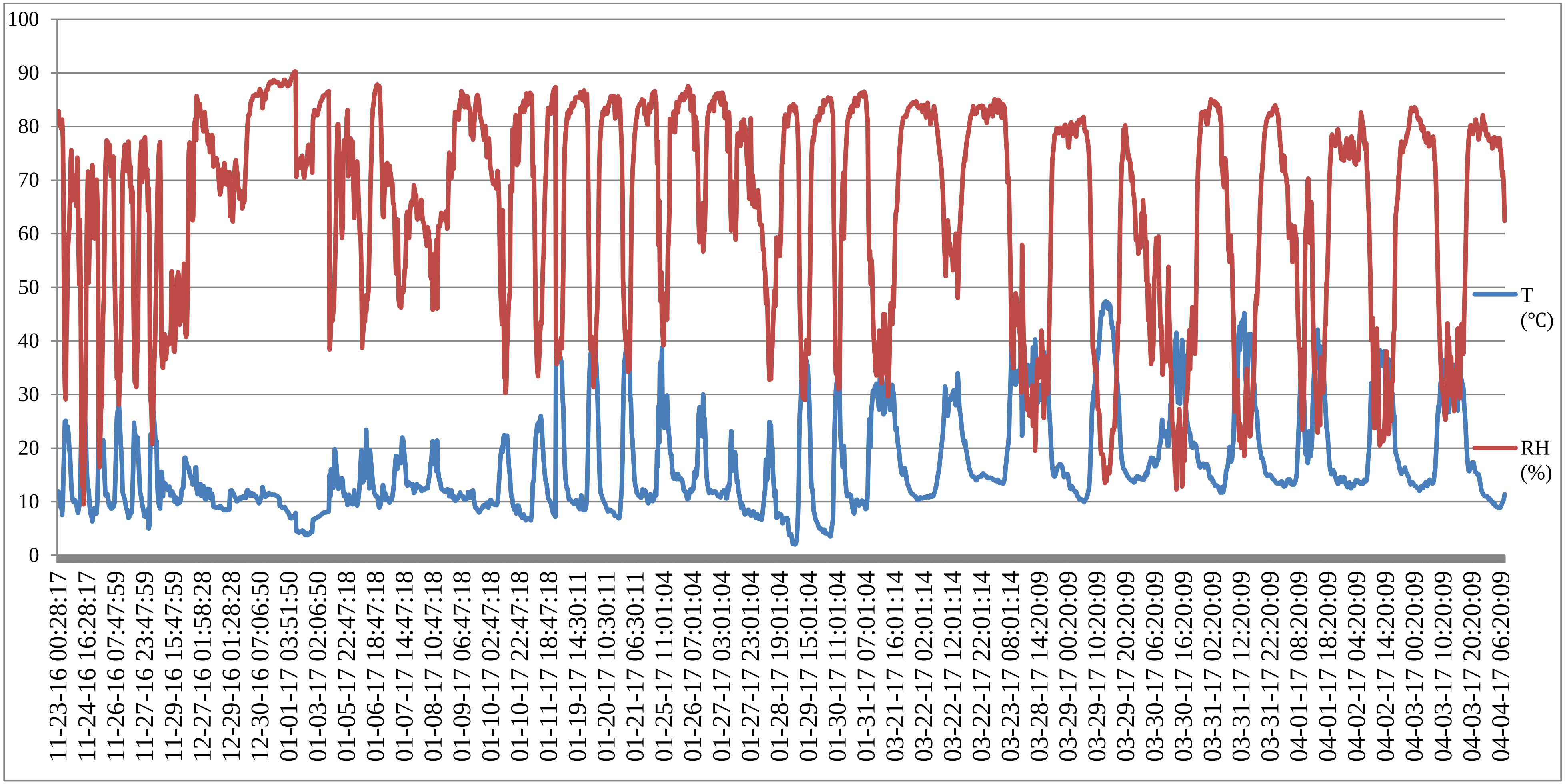
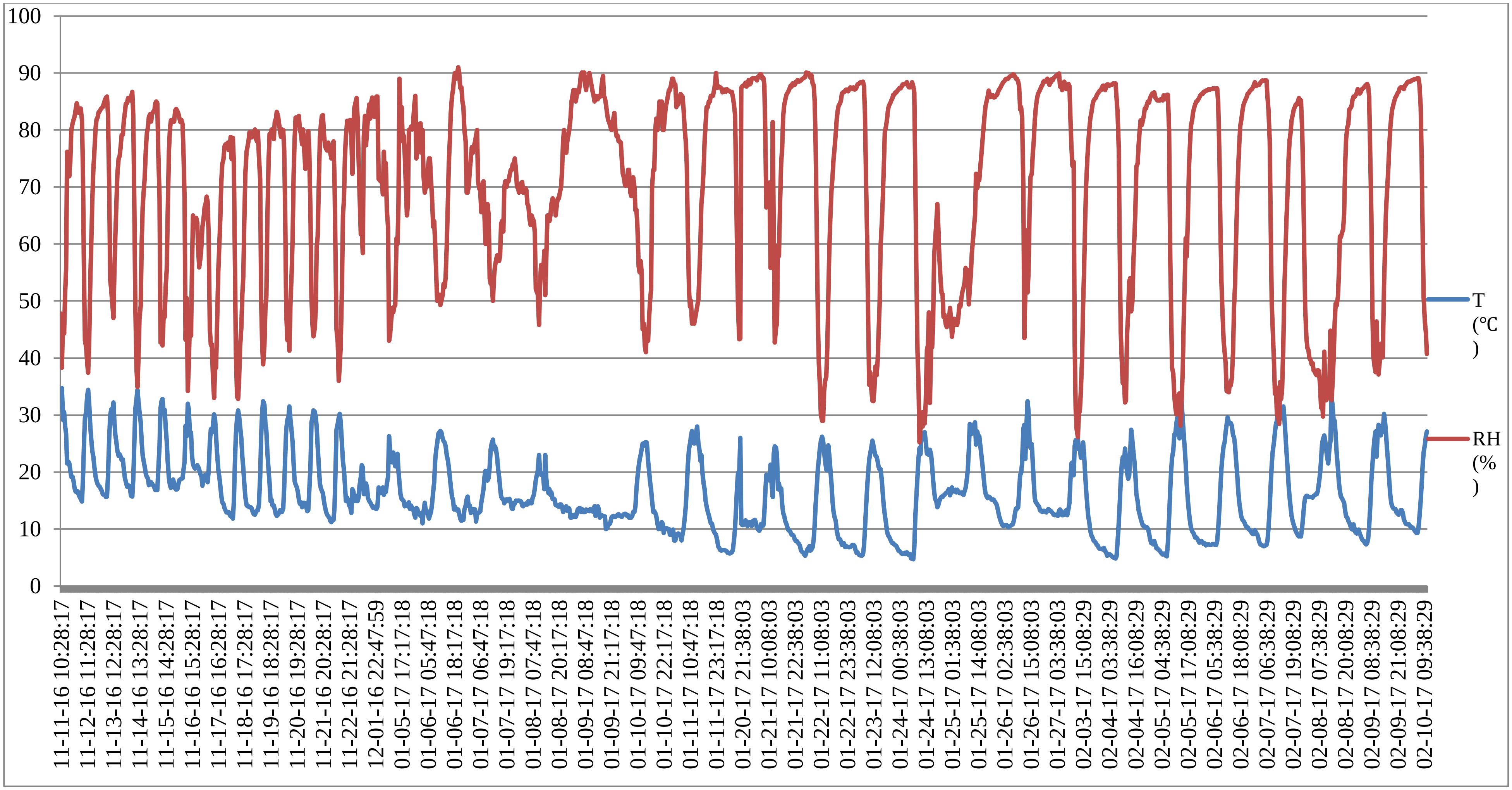


Figure II. Temperature and RH variations recorded throughout the growing period at site B



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