

AMERICAN UNIVERSITY OF BEIRUT

BIO-INTENSIVE INTEGRATED PEST MANAGEMENT  
(BIPM) OF GREENHOUSE VEGETABLE PESTS USING  
LOCAL STRAINS OF BEAUVERIA PSEUDOBASSIANA,  
AMBLYSEIUS SWIRSKII AND PHYTOSEIULUS  
PERSIMILIS

by  
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## ABSTRACT OF THE THESIS

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Major: Plant protection

Title: Bio-Intensive Integrated Pest Management (BIPM) of Greenhouse Vegetable Pests using Local Strains of *Beauveria pseudobassiana*, *Amblyseius swirskii*, and *Phytoseiulus persimilis*

Tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus* L.) are the most commonly produced vegetable crops in Lebanon in protected cultivation. However, their production has been seriously constrained by different factors including arthropod pests. Major arthropod pests include whiteflies, thrips, spider mites, aphids and leafminers; the management of which remains a challenge due to resistance development against synthetic pesticides. Given the limited studies on the effectiveness of bio-control agents in Lebanon, the objective of this research was to assess the control efficacy of locally collected entomopathogenic fungus *Beauveria pseudobassiana* Rehner and Humber (Hypocreales: Cordycipitaceae) and the predatory mites *Amblyseius swirskii* and *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae) for the management of greenhouse arthropod pests in two large-scale commercial greenhouse trials on cucumber and tomato. A third trial was conducted in insect-proof cages to assess the compatibility between *B. pseudobassiana* and *A. swirski* against *Bemisia tabaci* (Gennadius) (Hemiptera; Aleyrodidae). In field trials, three greenhouses were selected at Rmeileh area, South Lebanon. In the control greenhouse, the farmer followed his normal plant protection practices while the other two greenhouses were subject to IPM practices; IPM-A and IPM-B to evaluate the efficacy of *B. pseudobassiana* and the two local predatory mites, respectively. In the 1<sup>st</sup> trial on cucumber, the populations of *Frakliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) were maintained below ETL in the control, while that of *B. tabaci* exceeded the ETL and an outbreak among *Tetranychus urticae* Koch (Acari: Tetranychidae) occurred, forcing the farmer to stop the production cycle despite the application of six sprays of pesticide mixtures. In IPM-A, five *B. pseudobassiana* sprays showed the highest control level by suppressing *B. tabaci*, *F. occidentalis*, *T. urticae*, and *M. persicae* to below their respective ETL, allowing 100% reduction in pesticide applications. Four releases of *A. swirskii* and three hotspot treatments of *P. persimilis* in IPM-B successfully maintained *B. tabaci* and *F. occidentalis* below ETL, despite elevated temperatures (T. average 32°C and Max. 54-41 °C) recorded in September-October and (T. average 26°C and Max. 40-49 °C) in November. In the tomato trial, *Bacillus thuringiensis* var. kurstaki (Btk) was used as a microbial biocontrol agent instead of the predatory mites in IPM-B since *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was the major pest. In the control greenhouse, the farmer applied 20 pesticide sprays using 2-3 active ingredients per spray, yet obtained the lowest level of control against *T. absoluta*; 3.8 eggs/leaf and 3.6 larvae/leaf at the end of the trial. In IPM-A, five *B. pseudobassiana* sprays outperformed all treatments against *T. absoluta*; 0.4 eggs/leaf and 1.9 larvae/leaf; representing significant reductions of 89.5% among eggs and 47.2% among larvae as

compared to the control. Meanwhile, five applications of *B. thuringiensis* in IPM-B reduced the number of *T. absoluta* eggs and larvae by 80 and 27%, respectively. Both bio-control agents reduced leaf miner (*Liriomyza spp.*) populations by 80% with respect to the control. The small scale greenhouse experiment revealed 97.85% efficacy of *A. swirskii* against *B. tabaci* nymphs, which increased to 99.2% when combined with *B. pseudobassiana*, indicating high compatibility. *B. pseudobassiana* was also active against *B. tabaci*; 86.3% mortality rate. Overall, adopting biologically-integrated pest management (BIPM) for pest population in greenhouse cucumber and tomato production appears promising under Mediterranean environmental conditions.

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## ABBREVIATIONS

°C	Degree Celsius
g	Gram(s)
Kg	Kilogram(s)
L	Liter(s)
mL	Milliliter(s)
mg	Milligram(s)
%	Percent of a hundred
m <sup>2</sup>	Square meter
a.i.	Active ingredient
ANOVA	Analysis Of Variance
<i>A. swirskii</i>	<i>Amblyseius swirskii</i>
<i>B. bassiana</i>	<i>Beauveria bassiana</i>
<i>B. pseudobassiana</i>	<i>Beauveria pseudobassiana</i>
BSC	Blue sticky cards
<i>B. tabaci</i>	<i>Bemisia tabaci</i>
<i>B. thuringiensis</i>	<i>Bacillus thuringiensis</i>
CMV	<i>Cucumber mosaic virus</i>
<i>C. tannourinensis</i>	<i>Cephalcia tannourinensis</i>
EIL	Economic injury level
<i>et al.</i>	<i>et alia</i> (and others)
ETL	Economic threshold level
FAO	Food and Agriculture Organization
<i>F. occidentalis</i>	<i>Frankliniella occidentalis</i>

Hrs	Hours
IPM	Integrated Pest Management
L:D	Light to Dark ratio
M	Million
<i>M. persicae</i>	<i>Myzus persicae</i>
PDA	Potato Dextrose Agar
<i>P. persimilis</i>	<i>Phytoseiulus persimilis</i>
®	Registered trade mark
Spp.	Species
<i>S. tuberosum</i>	<i>Solanum tuberosum</i>
<i>T. absoluta</i>	<i>Tuta absoluta</i>
<i>T. urticae</i>	<i>Tetranychus urticae</i>
<i>T. vaporariorum</i>	<i>Trialeurodes vaporariorum</i>
WMV	<i>Watermelon mosaic virus</i>
WP	Wettable powder
YSCs:	Yellow sticky cards

# CHAPTER 1

## INTRODUCTION

The world is facing a global food security crisis (GNAFC, 2021). In addition to key drivers such as population growth, conflict, economic shocks and climate extremes, the COVID-19 pandemic exacerbated the challenge (FAO, 2021). A recent report by the World Bank (2021) revealed that nearly 2.37 billion people (or 30% of the global in one year. Evidence suggests that the ability to recover from the increasing adverse effects is now more difficult (GNAFC, 2021). In light of the present situation, there is an urgent need to implement serious long-term measures to reverse the current trajectory and increase food production.

A transformation in agriculture practices is needed to achieve resilient and sustainable food systems (FAO, 2021). Global food production must increase by 70% to keep pace with the growing demand while using the same amount of natural resources; land and water (Bruce, 2010). Pest management is a fundamental element in sustainable agriculture (Sorby et al. 2003). Among many factors that cause significant food losses, pathogens and insect pests are of concern (Shankar and Abrol, 2012). Up to 40% of the global crop production is lost annually due to pests (FAO, 2021). The pest pressure in protected crops is even more concerning due to favorable conditions, causing 30 to 40% yield loss, which in many cases reach up to 100% due insect-transmitted viral diseases (Shivalingaswamy et al., 2002). Major arthropod pests on vegetables; namely cucumber and tomato are 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 gossypii* Glover and *Myzus persicae* Sulzer) (Şovărel et al., 2019). Also, *Tuta absoluta* Meyrick is the most destructive pest of tomatoes. The control of such pests has relied for a long time on chemical pesticides, until consistent failures in field performance were reported due to rapid emergence of pest resistance. Ever since, a rise in yield loss was observed despite an increase in the global market of synthetic chemical pesticides and their use. Additionally, there is a rising public concern about the deleterious effects of chemical pesticides on consumer health, environment and biodiversity. In this context, the concept of integrated pest management became a robust paradigm as a holistic approach toward sustainable development. It is perceived as an environmentally-friendly way that utilizes multiple synergistic pest-control tools to provide safe and quality produce while minimizing health risks and conserving non-renewable resources (Shankar and Abrol, 2012). In practice, IPM may vary from being a chemically-based system that include careful usage of selected chemicals to biointensive integrated pest system (BIPM) that relies mainly on the use of natural enemies and biocontrol agents in pest control (Sorby et al., 2003). While protected greenhouse environments are advantageous for pest development, they can be maintained to favor biological control agents (BCAs), which are beneficial organisms that act on insect pests. BCAs include predators, parasitoids, pathogens and competitors. Predatory mites (Acari) are important components of IPM programs, being the second largest group pf BCAs (Van Lenteren 2016). Among these, *Phytoseiulus persimilis* and *Amblyseius swirskii* Athias-Henriot (Arachnida: Phytoseiidae) proved very successful. Likewise, entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin has been widely used against a variety of arthropod pests.

The objectives of this study are as follows:

1. Test the efficacy of the local fungus *Beauveria pseudobassiana* and locally collected predatory mites *Amblyseius swirskii* and *Phytoseiulus persimilis* for the integrated management of major tomato and cucumber pests under commercial greenhouse conditions.
2. Determine the compatibility of *B. pseudobassiana* and *A. swirskii* in the management of whiteflies, using small scale replicated insect-proof experiments.

## CHAPTER 2

### LITERATURE REVIEW

#### **2.1. Protected Cultivation in Mediterranean Region**

Protected cultivation emerged as a way to grow crops in a controlled environment, providing year-round, high-quality produce (Baudoin et al. 2013). Commercial greenhouse production appeared in Northern Europe during the early decades of the 20<sup>th</sup> century and expanded rapidly in many regions throughout the world, particularly the Mediterranean. Due to its mild winter climate and the possibility of adopting low cost, simple protective structures, the Mediterranean region became one of the leading and most competitive producers of greenhouse crops with a total area of 350,000 Ha in recent years (De Pascale & Maggio, 2004). More than 80% of these areas are devoted to vegetable production, predominantly being solanaceous crops (tomato, eggplant, pepper) and cucurbits (cucumbers, watermelons, melons, zucchini), for their high local consumption and export potential (Tuzel and Leonardi, 2009).

The use of greenhouses promotes sustainable crop production by optimizing the use of available resources. Major improvements in water-use efficiency were reported under greenhouse conditions due to lower evapotranspiration rates, which in return reduces fertilizer leaching (Baudoin et al. 2013). Growers are also able to control climatic fluctuations such as temperature, humidity and light intensity. A wide range of environmentally and economically sound pest control measures are implemented to limit pesticide applications such as the use of insect-proof nets, banker plants and biocontrol agents.

## **2.2. Greenhouse vegetable production in Lebanon**

The Lebanese Greenhouse industry is almost completely dedicated to vegetables with over 4,000 Ha in production (Habib et al. 2020). Most greenhouses are located across the coastal area of Mount Lebanon, North and South regions with tomato and cucumber being the most cultivated (Habib et al. 2020). Before the current economic crisis, greenhouse suppliers reported an increase in installation sales indicating an expansion of the sector (ILO, 2020).

### **2.2.1. Cucumber**

The family *Cucurbitaceae*, also known as cucurbits, is the most diverse plant family comprising 130 genera and 800 species. Within this family, major cultivated crops are pumpkins (*Cucurbita maxima* Duch), cucumbers (*Cucumis sativus* L.), melons (*Cucumis melo* L.) and watermelons (*Citrullus lanatus* (Thunb.) Matsumura and Nakai). In Lebanon, the cultivation of cucumber (*Cucumis sativus* L) is increasing continually as new production methods and plastic houses are used (FAO, 2002). Being an annual, cold sensitive crop that responds well to high humidity (80%) and warm temperatures (16-30 °C), cucumbers are widely grown under protected cultivation in Coastal Lebanon all year round. They are also grown in open fields of the Beqaa valley during late spring and summer (Fahed, 2005).

In 2019, the cultivated area of cucumber and gherkins reached 3,533 Ha with an annual production of 128,713 tons (FAOSTAT, 2019). Pest management remains heavily reliant on frequent pesticide applications which are highly residual in cucumbers as they are harvested every other day (Fahed, 2005).

### **2.2.2. Tomato**

Tomato (*Lycopersicon esculentum*) is the second most grown vegetable in Lebanon and the major crop in protected cultivation (MoE/UNDP, 2011). It is cultivated in all regions with varying climatic conditions either in greenhouses or open fields. Tomato is a warm-season crop that is severely affected by temperature extremes, frosts and high humidity. Although it is perennial, tomato is grown as an annual crop in temperate areas due to its sensitivity to temperatures below 12°C (Peet & Welles, 2005). Damage in plant tissues was observed at temperatures less than 10 °C and above 38 °C (Naika et al. 2005). Most varieties maintain an optimal growth between 21 °C and 24 °C (MoE/UNDP, 2011).

Data on tomato production shows an annual yield of 300,157 tons on a harvested area of 3,810 ha (FAOSTAT, 2019). Multiple growing seasons of tomato persist, ensuring its year-round availability in the Lebanese market. In the coastal area of Lebanon, tomato is grown in greenhouses for at least two growing seasons per year: the first one during July-August or October-November, and the second starting anywhere between November and February (MoE/UNDP, 2011). In moderate altitudes like the Beqaa valley (500-1200 m) and high altitudes of Mount Lebanon (1200-200 m), production is limited to one season as frost incidences are likely. However, the intensification of greenhouse tomato production created ideal conditions for pest development leading to high yield losses (Naika et al. 2005).

## **2.3. Major greenhouse pests of Cucumber and Tomato in Lebanon**

### **2.3.1. Whiteflies**

#### **2.3.1.1. Classification**

The Aleyrodidae family comprise 1556 whitefly species within 120 genera. The insects are 1-3 mm long in the order Hemiptera. In Lebanon, the sweet potato whitefly *Bemisia tabaci* is a serious global pest of greenhouse tomatoes and cucumbers (Sani et al. 2020). *Bemisia tabaci* is a complex of genetically diverse species comprising at least 44 discrete types (Bello et al. 2020). The most invasive and widely distributed strains are the Middle East-Asia Minor 1 (MEAM1, B biotype) and Mediterranean (MED, Q biotype) causing substantial economic losses in the global greenhouse vegetable industry estimated around 10 billion US dollar (USD) from the year of 1980 to 2000 (De Barro et al. 2011; Horowitz et al. 2020).

#### 2.3.1.2. Host range

Damage is caused either directly through the pest polyphagous feeding habit (more than 700 species from 86 families), or indirectly through the transmission of more than 200 viral diseases of which is *Tomato yellow leaf curl virus* (TYLCV) (Greathead, 1986; Sani et al. 2020). Adults and immatures feed on phloem sap and secrete relatively high amounts of ‘honeydew’ on leaves and fruits which favors the development of fungal growth (black sooty mold), interfering with the photosynthetic activity, thus reducing yield and quality (Horowitz et al. 2020).

#### 2.3.1.3. Life cycle

The life cycle of whiteflies consists of six stages: the egg, four larval stages and adult stage. Though the insect has a high fecundity rate, its development time from egg to adult take anywhere between 16 to 31 days depending on several factors such as relative

humidity, temperature and host plant (Sani et al. 2020). A study by Sohani et al. (2004) showed that the mean development rate and egg production of *B. tabaci* on cucumbers were optimum at 30°C being 14.1 days and 204.7 eggs respectively. Likewise, peak egg production of 203.1 eggs at the same temperature was reported on tomatoes (Hendi et al. 1984).

#### 2.3.1.4. Pest management

Chemical control is the main approach adopted by farmers to control whiteflies. Historically, heavy reliance on conventional insecticides has led to the development of insect resistance to most chemical classes including organophosphates, carbamates, pyrethroids, insect growth regulators (pyriproxyfen and buprofezin) and neonicotinoids (Sani et al. 2020). More recently, resistance against the class of ketonolols that inhibit lipid biosynthesis was documented in Spain and India (Fernandez et al. 2009; Roy et al. 2019). Lately, Wang et al. (2018) reported low/moderate resistance in the MED strain of *B. tabaci* to a diamide insecticide; cyantraniliprole (Nauen and Steinbach, 2016). Until now, 673 cases of insecticide resistance in 65 different active ingredients are described within the genus *Bemisia* (Arthropod Pesticide Resistance Database, Michigan State University). The implementation of alternative control methods is essential to keep the pest below economic injury levels of 4 nymphs per tomato leaf or 5 adults per cucumber leaf (Gusmão et al. 2006; Jeon et al. 2009).

## **2.3.2. Thrips**

### 2.3.2.1. Classification

Thrips (Thysanoptera: Thripidae) are elongate, small sized insects of only few millimeters or less in length. Nearly 1 % out of 5500 globally described species are identified as invasive pests of commercial crops (Morse et al. 2006). In protected cultivation of vegetables, major species are the western flower thrips, *Frankliniella occidentalis*, the onion thrips, *Thrips tabaci* and the melon thrips, *Thrips palmi*.

### 2.3.2.2. Host range

After originating in the 1970s, intensive studies showed that *F. occidentalis* is the dominant and most widely spread species in at least 57 countries (He et al. 2020). The pest shows a cryptic behavior and is highly polyphagous with a broad host range including 250 crops within 62 families (Lewis et al. 1997). In addition to its extensive feeding and oviposition damage on vegetables and fruits, *F. occidentalis* is an efficient vector of 11 viruses so far, 8 of which belonging to the genus *Tospovirus* including the most economically significant virus, *Tomato spotted wilt virus* (TSWV), infecting 1090 plant species from 85 families (Campbell et al. 2008).

### 2.3.2.3. Life cycle

*F. occidentalis* exhibits a high reproductive capacity with a short life cycle consisting of the egg, two larval stages, pre-pupal and pupal stage and adult. Optimal development occurs at 25-30°C (Reitz et al. 2019). The economic injury level of *F. occidentalis* was established at 3 to 7.5 adults per cucumber flower and 1.3 adult or 9.5 larvae per cucumber leaf (Shipp et al. 2000; Jarošík et al. 1997).



#### 2.3.2.4. Pest management

Over years, heavy reliance on major insecticidal classes to control *F. occidentalis* led to resistance development and facilitated the pest's spread over other species in several countries (He et al. 2020). A practical way to cope with insect resistance is to use chemicals only when necessary within a complete IPM program (Gao et al. 2012).

### **2.3.3. Two-spotted spider mite**

#### 2.3.3.1. Classification

Mites make up a diverse group of arthropod pests belonging to the class of Arachnida and subclass Acari. Spider mites (Acari: Tetranychidae) are major pests of open field and greenhouse crops (Marić et al. 2017). The two-spotted spider mite, *Tetranychus urticae* Koch, is the most serious and consistent pest in the family Tetranychidae.

#### 2.3.3.2. Host range

*Tetranychus urticae* is known to affect a wide range of economic crops including vegetables, fruits and ornamentals. High mite populations caused extensive yield losses in greenhouse tomatoes and cucumbers (Park and Lee, 2005, Meck et al. 2013). *Tetranychus urticae* is reported to attack 3877 plant species as it feeds on the underside of the leaf tissues, thereby reducing chlorophyll content and photosynthetic rate (Park and Lee, 2005; Migeon and Dorkeld, 2007).

#### 2.3.3.3. Life cycle

The life cycle of spider mites is composed of 5 stages, eggs, larval/nymphal, protonymph, deutonymph and the adult stage (Van Leeuwen et al. 2009). Under favorable conditions, the mite develops rapidly with a short life cycle, high reproductive potential and long survival capacity (Clotuche et al. 2011). Optimal growth was observed at a temperature range of 20-30°C and low RH. A complete development of the mite took 6.5 days at 30°C on cucumber, and was retarded below 12°C or above 40°C (Meck et al. 2013).

#### 2.3.3.4. Pest management

Like other arthropod pests, the two-spotted spider mite is mainly controlled by acaricides. Over time, the pest was capable of building up very fast and high resistance levels to acaricides within 1-4 years of their introduction and use (Van Leeuwen et al. 2009). A total of 552 resistance cases are documented against 96 active ingredients, showing the highest prevalence of *T. urticae* among all other arthropod pests. Global resistance to abamectin, pyridaben, fenbutanin oxide, fenpyroximate and bifentazate was frequently reported (Arthropod Pesticide resistance Database, Michigan State University, 2021).

### **2.3.4. Aphids**

#### 2.3.4.1. Classification

Aphids (Hemiptera: Aphididae) are phytophagous insects encompassing more than 5000 species (Shuo et al. 2020). Of these, about 100 species evolved and exploited a wide range of crops, exhibiting serious threat to agriculture (Blackman and

Eastop, 2007). These pests are exceptional for their reproductive polyphenism that provides them with phenotypic plasticity to adopt distinct phenotypes in response to environmental variations (Shuo et al. 2020). In greenhouses, *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas, and *Myzus persicae* Sulzer are the most destructive to vegetables (Thrope et al. 2016).

#### 2.3.4.2. Host range

The green peach aphid, *Myzus persicae* (Sulzer), is a serious aphid crop pest due to its cosmopolitan distribution, life cycle, host range, capacity to disperse, ability to transmit viruses and to develop insecticidal resistance (Bass et al. 2014). It is highly polyphagous, with a host range of over 400 plant species from 50 different families (Silva et al. 2012). Damage is either caused by feeding on plant sap and honeydew secretion or by viral transmission. *Myzus persicae* is an efficient vector of more than 100 plant viruses that make up 50% of the most damaging insect transmitted viral diseases like cucumber mosaic virus (CMV) and watermelon mosaic virus (WMV) (Perdikis et al. 2008).

#### 2.3.4.3. Life cycle

Aphid's reproduction includes both the parthenogenetic and sexual generations (Rousselin et al. 2017). During the growing season in greenhouses, aphids continuously give birth to via viviparous reproduction without mating. In the fall season, as the day length becomes short, winged male aphids develop in colonies and mate with the females, which then produce eggs. The eggs overwinter on perennial plants and hatch in the spring.

Later on, they fly to new plants and can be transported very long distances in air currents to infest other crops (Flint, 2013).

#### 2.3.4.4. Pest management

*Myzus persicae* has been almost exclusively controlled by insecticides, allowing for the development of multiple resistance forms by different methods of metabolic mechanisms and direct site mutations (Silva et al. 2012). The first case of resistance was reported in 1995, with a total of 475 resistance cases to date within most chemical classes including organophosphates, pyrethroids, carbamates and neonicotinoids (Foster et al. 2007, Mota-Sanchez and Wise, 2021). Therefore, the integration of novel control means is required for the management of aphids. However, the decision to apply control measures for aphids is dependent on measurable thresholds (economic injury level and economic threshold). Very limited information is found on the economic thresholds of aphids on vegetables. A study done in South Korea estimated the economic threshold level (ETL) of *Myzus persicae* on Chinese cabbage based on a developed economic injury level (EIL) model: 20 aphids per plant (Jeon et al. 2008). However, in the absence of well-established economic thresholds, adequate monitoring of aphids growth rate is very crucial within the first few days since the pest can build up very rapidly, and even at low populations, transmit viral diseases.

#### **2.3.5. *Tuta absoluta***

##### 2.3.5.1. Classification

*Tuta absoluta* (Meyrick) is a Lepidopteran insect belonging to the family Gelechiidae. Originating in South America, *Tuta absoluta* was able to spread throughout

Europe, the Mediterranean Basin, Africa and some countries in Asia within 12 years only, and is now reported in more than 80 countries (Zhang et al. 2021). It is a key pest of open field and greenhouse tomato worldwide. In Lebanon, the first records of *T. absoluta* were in April 2010 in the coastal area of Jbeil and it was later spread all over the country causing serious damage in open field and protected tomato.

#### 2.3.5.2. Host range

Besides tomato, the pest attacks other solanaceous crops and weeds as well as plants from different other families (Asteraceae, Poaceae, and Fabaceae) (Ferracini et al. 2019). The mining-feeding behavior of the larvae on all parts of the crop, mainly leaves and fruits of young plants cause severe damage reaching up to 100% yield loss in the absence of proper management.

#### 2.3.5.3. Life cycle

The tomato leaf miner is a multivoltine species with a high reproductive capacity. Under favorable conditions, a female can lay up to 300 eggs with 10-12 generations per year (Biondi et al. 2018). Eggs are laid in upper plant parts on young leaves, stems, or sepals (Cocco et al. 2015). The larva feeds by mining the leaf mesophyll, thus producing a thin leaf mine. At high densities, larvae penetrate axillary buds of young stems or tomato fruits (Desneux et al. 2010). Mature larvae usually drop to the soil where they produce a thin, silky cocoon to transform into prepupae and pupae. Adult mating relies on female sexual pheromones, and mating lasts from a few minutes up to six hours (Lee et al. 2017).

#### 2.3.5.4. Pest management

Several control measures are practiced to control *T. absoluta* either solely or within an integrated pest management program. Yet, farmers in many countries still rely on heavy applications of insecticides. It has been reported that one production season of tomato requires up to 5 sprays per week and 36 sprays in total which may still be insufficient (Ferracini et al. 2019). Hence, fast and global resistance levels to major classes of insecticides including organophosphates, pyrethroids, pyrethrins, spinosyns, carbamates, diamides, and oxadiazines (Biondi et al. 2018; Guedes et al. 2019; Bala et al. 2019). Indeed, such trends led to the development of pest resistance and sublethal effects on non-target organisms such as natural enemies and pollinators (Guedes et al. 2019).

Multiple Biological control agents including invertebrates and microbial organisms have been evaluated for their efficacy in reducing *T. absoluta* population. For instance *Macrolophus pygmaeus* Rambour and *Nesidiocoris tenuis*; two mirid bugs preying on egg and larval stages of *T. absoluta*, were found to be successful in reducing leaf and fruit damages (Desneux et al. 2010). Microbial control relies mostly on commercial strains of *Bacillus thuringiensis* (*Bt*) var. *kurstaki* and *aizawai* that kill larvae when ingested (González-Cabrera et al. 2011). Entomopathogenic fungi have been used as well including *B. bassiana* and *M. anisopliae* (Borgi et al. 2016; Contreras et al. 2014) but no commercial products are specifically designed for *T. absoluta* yet.

### **2.3.6. Leaf miner**

#### 2.3.6.1. Classification

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).

#### 2.3.6.2. Host range

Major crop hosts include cucurbits, solanaceous and leguminous crops along with many others crops (Weintraub & Horowitz, 1995). Feeding punctures and leaf mines are usually the first and most obvious sign of the presence of *Liriomyza* spp to due to larval leaf mining. Potential damage include 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).

#### 2.3.6.3. Life cycle

The life cycle of *Liriomyza* spp. comprises: egg, three larval instars, pupae and adult stage. The average period of the life cycle of the celery leaf miner is 21 days, but can be as short as 15 days. The length of the life cycle varies with host and temperature (Gao et al. 2017).

#### 2.3.6.4. Pest management

The control of the leaf miner traditionally depended on chemical insecticides; however, in many countries, significant levels of resistance to all major classes of insecticides appeared. Application of insecticide with different modes of action is recommended when necessary (Gao et al. 2017). Biological control of leaf miners has

been proposed including natural enemies (wasps and parasitoids), entomopathogenic nematodes and fungi as well as microbial bio pesticides such as *B. thuringiensis*.

#### **2.4. From Chemical control to integrated pest management (IPM)**

During the 1940s and 1950s, crop pest management relied primarily on chemical control due to its efficacy, convenience and low cost (Kaur and Kaur, 2020). By time, the persistent overuse of pesticides led to the evolution of pest resistance, environmental toxicity, negative side-effects on non-target organisms and consumer health, as well as a decrease in food quality and crop yield. Thus, stringent pesticide registration procedures were introduced by governments, promoting the adoption of integrated pest management (IPM) as a sustainable and ecofriendly alternative paradigm. IPM is a holistic approach that combines all available control methods to maintain pests below economic threshold levels while keeping the use of pesticides to a level that is economically justified and minimizing overall human health and environmental risks. According to FAO (2013), IPM is a pillar of both pesticide risk reduction and sustainable crop production. It constitutes of general basic principles like prevention, pest identification, periodic monitoring and inspection, determination of economic and action thresholds levels, implementing a wide array of pest control measures and lastly, evaluation and record keeping (Kaur and Kaur, 2020).

Often perceived as the cornerstone of IPM, biological control is the oldest and perhaps most well-researched non-chemical element of IPM systems. It is defined as the use of beneficial organisms (parasitoids, predators and pathogens) to control pests (Van Lenteren et al., 2016). Biological control is classified based on three basic forms: classical, conservation and augmentative control. The latter approach involves mass



rearing and periodic releases of large numbers of natural enemies to provide sufficient pest control.

## **2.5. Biological control in greenhouses**

Biological control of arthropod pests in greenhouse crops has been successfully applied for decades and is likely to rise overtime as growers aim to reduce the impacts of pesticide resistance (Gonzalez et al. 2016; Pilkington et al. 2010). While protected cultivation may be more susceptible to pest development compared to open fields, greenhouses provide a distinctive niche for the biological control of plant pests (Perdikis et al. 2008). They are isolated units that are cleansed and kept pest free from previous crops. During the growing season, isolation provides a barrier for pest immigration to crops and dispersal of natural enemies. The climate control in greenhouses allows for optimizing conditions of bio control agents (Gonzalez et al. 2016).

The first use of biological control in protected cultivation dates back to 1927 in the UK for whitefly management using the parasite *Encarsia formosa* Gahan. After being largely produced and distributed throughout Europe and other countries, the parasite was discontinued as new insecticides provided sufficient control in greenhouses after 1945 (Van Lenteren et al. 1992). Few years later, *Tetranychus urticae* developed resistance to a number of acaricides which revived research in biological and discovery of *Phytoseiulus persimilis* as an efficient predator of spider mites. The number of natural enemies grew rapidly with the development of effective mass-rearing and introduction systems. Currently, around 500 commercial companies and 200 manufacturers produce at least 350 invertebrate biocontrol agents and 94 different species of microbial agents, respectively (Van Lenteren et al. 2018). More than 90% of

natural enemies belong to the Arthropoda within which are three major taxonomic groups namely: Hymenoptera (>50% species), Acari (15%) and Coleoptera (12%) (Van Lenteren et al. 2020). Predatory mites such as *Phytoseiulus persimilis*, *Amblyseius swirskii*, *Neoseiulus californicus*, and *Neoseiulus cucumeris* had an enormous contribution to the market growth of biocontrol agents as they proved successful against whiteflies, thrips and phytophagous mites. Since a large number of biocontrol agents have been used, this review will focus on the three biocontrol agents used in this study.

### **2.5.1. The predatory mite *Amblyseius swirskii***

#### **2.5.1.1. Classification**

Originating from the East Mediterranean coast, generalist predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) also called *Typhlodromips swirskii* is one of the most efficient biocontrol agents currently being used in over 50 countries (Calvo et al., 2015). The Phytoseiidae family is divided into four categories according to their feeding habits (McMurtry & Croft, 1997). Type I mites are specialized predators of spider mites, mostly *Tetranychus* spp. Type II species feed on spider mites, other small mites, pollen and even on plant exudates. Type III, are generalists that often prefer prey other than spider mites such as thrips. Type IV phytoseiid mites are members of *Euseius* specie; generalist predators that develop and reproduce best on pollen. Among the most commonly used mites in greenhouses are *A. swirskii* (type III) and *Phytoseiulus persimilis* (type I).

#### 2.5.1.2. Morphology

Eggs are oval-shaped and pale-whitish whereas immature stages are more transparent. Adults are pear-shaped, 0.5 mm in length with an unsegmented body and four pairs of legs.

#### 2.5.1.3. Host range

The mite controls primarily the egg and nymphal stages of the whiteflies *B. tabaci* and *T. vaporariorum* and the larval stages of the thrips *F. occidentalis* (Calvo et al. 2012). Being a generalist predator, *A. swirskii* is capable of preying on spider mites and broad mites (Messelink et al., 2006). In the absence of prey, it can develop and reproduce on non-prey food sources like pollen and nectars of different plants allowing it to persist in the crop (Calvo et al. 2015). The mite can also feed readily and survive on honeydew, although development is retarded and oviposition stops (Ragusa and Swirskii, 1977). Therefore, honeydew can serve as a supplement in the presence of other prey or as survival diet when sufficient food is limited.

#### 2.5.1.4. Life cycle

Development of *A. swirskii* depends on the type and availability of food source as well as the environmental conditions. According to Momen and Elsaway (1993), as the mite feeds on prey compared to pollen, it maintains higher oviposition and faster development rates. The life cycle of *A. swirskii* is composed of five stages: egg, larva, two nymphal stages and adult. It develops normally at a wide temperature range of 15.5°C to 37°C with at least 60% RH (Calvo et al. 2015). Rapid growth occurs between 20 °C and 32 °C with an optimal temperature of 30.5 °C (Lee et al. 2011). According to

Xu and Enkegaard (2010), *A. swirskii* consumes spider mite nymphs with a predation rate of 4–6 nymphs within a 12 h period, and twice the amount when preying on thrips. Immature mites consumed 15-20 whitefly eggs or nymphs, whereas adults fed on 2 eggs or 3 nymphs per day (Perring et al. 2018). *The mite can establish* on a wide range of greenhouse vegetables, some ornamental crops (chrysanthemum and roses), and fruit trees like citrus. It was capable of successfully suppressing *B. tabaci* and *F. occidentalis* populations on cucumber, sweet pepper, eggplant and melon in Spain (Calvo et al. 2009). However, the phytoseiid mite cannot provide effective control on tomato plants due to the presence of high concentrations of trichome secondary metabolites.

#### 2.5.1.5. Pest control

Under greenhouse conditions, the recommended release rate of *A. swirskii* varied between 25 and 100 mites per m<sup>2</sup> depending on the pest species, pest densities and crop type (Dogramaci et al. 2013). Upon its release within an IPM program, the mite can be simultaneously supported with selective compatible pesticides; chlorantraniliprole, pymetrozine and flonicamid, and microbial insecticides like the pathogenic fungus *Beauveria bassiana* and *Metarhizium anisopliae* at high infestation levels (Brownbridge and Buitenhuis, 2019, Dader et al. 2020).

#### 2.5.1.6. Local strain

A Lebanese isolate of *A. swirskii* was collected from Batroun area. The mite isolate was first classified based on its morphological characteristics. Later on, molecular identification of the mite was done at the plant pathology lab at AUB-FAFS. Based on the PCR, cloning and sequencing results of the ITS gene, a 99% similarity

was found in the ITS nucleotide sequence as compared to published *A. swirskii* cultures at NCBI using Blast program.

### **2.6.1. The predatory mite *Phytoseiulus persimilis***

#### 2.6.1.1. Classification

The most effective bio-control agent of the two-spotted spider mite *T. urticae* is the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae), a type I predatory mite (Takafuji and Chant, 1976). Among all marketed natural enemies, *P. persimilis* is the most available, corresponding to 12% of the total world market (Van lenteren, 2006).

#### 2.6.1.2. Morphology

The phytoseiid mite adult is pear-shaped, orange to red in color, and with a length of almost 0.5 mm. Eggs and immature stages are characterized by a pale orange color.

#### 2.6.1.3. Host range

*Phytoseiulus persimilis* is a specialist predatory mite used against spider mite species on vegetables and ornamentals. The mite has higher mobility compared to *T. urticae*. After sensing the volatile chemicals emitted by the attacked crop, *P. persimilis* adults detect their prey and feed on all life stages, while nymphs consume eggs, larvae and protonymphs (Takafuji and Chant, 1976). As a specialist predator, the mite will starve in absence of prey, and will undergo cannibalism or disperse from the field searching for alternative food (Malais & Ravensberg, 2004; DeBoer et al. 2004).

#### 2.6.1.4. Life cycle

The lifecycle of *P. persimilis* is similar to that of *A. swirskii*, consisting of five stages, namely: egg, larva, two nymphal stages, and adult stage. Under favorable conditions, females are able to lay 4 eggs per day and consume 30 eggs or 24 immature mites (Hussey et al. 1969). *Phytoseiulus persimilis* adult consumes 20 eggs, 20 larvae, or 5 adult spider-mites per day at a recommended rate of 12.5 mite/m<sup>2</sup> (Abou Haidar, 2018). The mite remains active year-round in greenhouses upon the availability of spider. Growth and pest control potential of *P. persimilis* are dependent on temperature and relative humidity (Skirvin and Fenlon, 2003). The mite population increases fast at a temperature range of 15°C to 27 °C and 60% to 90% RH. While optimal activity occurred at 27 °C and 60% -85% RH, it declined at temperatures > 30 °C or RH< 60% favoring the development of prey (Ravensberg et al. 1983). Conversely, a study by Rasmy and Ellaithy (1988) reported the first survival of *P. persimilis* and its sufficient control of *T. urticae* in greenhouse conditions in Egypt at temperatures rising over 35 °C in hot days.

#### 2.6.1.5. Pest control

The efficiency of *P. persimilis* is correlated with the predator-prey release ratios and the timing of mite introduction (Yanard et al. 2019). High release ratios or early introductions may result in the rapid decline of spider mite populations before the establishment of sufficient populations of *P. persimilis*. On the other hand, low release ratios or delayed introductions will lead to high spider mite infestations (Stravrinides, 2010). In greenhouse-grown cucumbers, *P. persimilis* provided adequate control of *T. urticae* at a moderately low predator: prey release ratio of 1:15 (Yanard et al. 2019).

Releasing *P. persimilis* 6 days post spider mite infestation at a rate of 5 mites/plant resulted in the highest number of mobile mites on greenhouse beans compared to a rate of 10 or 20 mites per plant (Stravrinides, 2010). In case of severe infestations, compatible acaricides are applied such as bifenazate and cyflumetofen (Atef et al. 2019). Unlike other vegetables crops, *P. persimilis* was reported to perform poorly on tomatoes due to toxic chemicals found in trichome exudates (Simmons and Gurr, 2006). Nonetheless, evidence suggests that the rearing of the mite on tomato plants for 4 generations can enhance its functional performance on tomato (Drukker et al. 1997). Practically, Tiftikci et al. (2020) found that *P. persimilis* successfully suppressed *T. urticae* population in second season tomatoes in Turkey at a release ratio of 1:10 and 1:20).

#### 2.6.1.6. Local strain

The *Phytoseiulus* strain was collected from bean plants grown in a conventional greenhouse in Jiyeh region- South Lebanon. The isolate was identified by morphologically and later by and molecular means at the plant pathology lab at AUB. PCR reactions were carried on the ITS region, followed by cloning and sequencing. The obtained nucleotide sequences were compared to the published sequences of *P. persimilis* at NCBI.

## ***2.7.1. The Entomopathogenic fungus Beauveria sp***

### 2.7.1.1. Overview

The entomopathogenic *Beauveria* sp. is a naturally occurring soil-borne fungus characterized by a cosmopolitan distribution and a wide host range. It is a facultative necrotrophic fungus that evolved unusual parasitic lifestyles, both as a saprophyte and a plant endophyte. Being initially described as *Botrytis bassiana* (Balsamo 1835), the species *Beauveria bassiana* was discovered in 1835 by Agostino Bassi as the infectious agent responsible for the white muscardine disease of silkworms that caused great losses in Italy and France (Redaelli and Visocchi, 1940). Following its discovery, several trials examined the potential of the fungus as a pest control agent until it became one of the most widely used commercial biocontrol agents. Several factors contribute to the success of *B. bassiana* in IPM systems. The fungus can be found in all soil types and is known to infect 707 insect species from 15 orders as well as 13 mite species. It exhibits different modes of virulence on different hosts which lowers the possibility of pests to develop resistance. The ability of *Beauveria* to attack insects by directly breaching the cuticle rather than being ingested is a great advantage against phloem feeding pests. Moreover, the fungus isolation, culturing, production and long term storage are relatively easy and cheap. Finally, its application has a limited effect on non-target organisms and beneficial insects. (Dannon et al. 2020).

### 2.7.1.2. Taxonomy, Morphology and Classification

*Beauveria* sp. was long described as a strictly anamorphic fungus reproducing by means of conidia. It was classified within the Deuteromycota, under the class Hyphomycetes, order Moniliales, and family Clavicipitaceae (Boucias & Pendland,



2012). However, a teleomorph stage was later identified by DNA-sequencing confirming the sexual reproduction of the fungus and placing it under phylum Ascomycota; class: Sordariomycetes; order: Hypocreales; family: Cordycipitaceae (Rehner et al. 2011).

Recent molecular classifications of *Beauveria* identified 26 distinct species including the most reported and widely registered as mycoinsecticides to control arthropod pests, *B. bassiana* and *B. brongniartii* (Rehner et al. 2011; Bustamante et al. 2019). Additional species such as *B. australis*, *B. pseudobassiana* and *B. sungii* serve as valuable biological control agents as well.

#### 2.7.1.3. Infection mechanism

Generally, *Beauveria* spp. infect their host by directly entering through the insect cuticle. Fungal infection begins when conidial spores that are initially dispersed by wind, rain or arthropod vectors attach to the host cuticle by chemical and electrostatic interactions (Pedrini et al 2013; Mascarin and Jaronski, 2016). Under suitable conditions, the spores germinate forming a germ tube through which the fungus, with the help of protein degrading enzymes (e.g., proteases, chitinases, amylases and lipases), penetrate the insect skin passing through all the cuticle layers until it reaches the hemolymph (Sanjaya et al. 2015). In the hemolymph, hyphal bodies multiply and spread through the insect body which becomes prone to the secretion of toxic secondary metabolites such as beauvericin, oosporein and bassianolide. These compounds act as immunosuppressants, colonizing internal tissues and depleting host nutrients (Mannino et al. 2019). They have also been correlated with antifungal and antimicrobial properties that inhibit the development of any saprophytic fungi and bacteria (Barra-Bucarei et al. 2020). Eventually, the host dies and conidiophores reemerge after several days showing

mummified cadavers that bear new infective conidia for future dispersal. *Beauveria bassiana* has also displayed potential evidence to invade its host through oral/intestinal infection routes (Maninno et al. 2019).

The virulence of *B. bassiana* is intrinsically influenced by biotic factors such as crop type, and host stage. For instance, the exposure of 3<sup>rd</sup> instar nymphs of *T. vaporarium* reared on cucumber plants to *B. bassiana* showed significantly higher mortality rates (95%) compared to the ones reared on tomatoes (38.7%) (Poprawski et al. 2000). Immature stages of the pest are known to be more susceptible to infection compared to adults as well (Abdo, 2008).

Likewise, temperature and humidity are key factors determining the efficacy of entomopathogenic fungi. Generally, *B. bassiana* is able to grow within a wide temperature range between 8-35°C (Fargues et al. 1997). The results of determining optimal thermal requirements for the growth of 65 different isolates of *B. bassiana* revealed a temperature range between 20°C to 30°C with most isolates falling between 25°C to 28°C. At temperatures between 23°C -25 °C, highest infection levels of adult *F. occidentalis*, *T. vaporariorum* and *A. gossypii* were obtained at 89% RH while that of *M. persicae* was at 97.5% RH (Shipp et al. 2003).

#### 2.7.1.4. Local *Beauveria* isolate

A naturally occurring Lebanese isolate of *Beauveria* was originally discovered from a dead third larval instar of cedar web-spinning sawfly, *Cephalcia tannourinensis* Chevin (Hymenoptera: Pamphiliidae) in a cedar forest of North Lebanon at an altitude of almost 1600 m (Abdo et al. 2008). The local isolate was initially classified based on conidial morphology which showed overlapping features between *B. bassiana* clade C and *B.*

*brongniartii* clade B (Abou-Jawdah et al. 2008). Accordingly, molecular based-phylogenetic analysis were performed for an adequate identification. The sequencing of multiple genes including the ribosomal internal transcriber spacer (ITS), nitrogen reductase, elongation factor 1-alpha (EF-1a) and DNA lyase revealed that the local *Beauveria* isolate is highly related to the members of clade C, referred to as *B. cf. bassiana* and later as *B. pseudobassiana* (Rehner and Buckley 2005; Abdo et al. 2008; Abou-Jawdah et al. 2008; Rehner et al. 2011).

Prior research studies agree that *B. pseudobassiana* is a successful bio-control agent. Under laboratory conditions, significantly higher mortality rates of the pine processionary moth, *Thaumetopoea wilkinsoni*, were observed with treatments of *B. pseudobassiana* compared to Diflubenzuron (Dimilin SC48) treatments. Similarly, effective control was reported against the pea leafminer, *Liriomyza huidobrensis* pupae (Noujeim et al. 2015). Another study conducted by Bedini et al, (2018) under both *in vitro* and *in planta* conditions showed that roughly all stages of the medfly, *Ceratitis capitata*, (Diptera Tephritidae) are highly susceptible to *B. pseudobassiana*. Similar results were reported by Alvarez-baz et al., (2015) when sawyer beetles *Monochamus galloprovincialis* (Olivier) (Coleoptera: Cerambycidae) were sprayed with the fungus.

#### 2.7.1.5. Mass production of *Beauveria*

A key factor for obtaining an efficient mycopesticide is the availability of cost-effective mass production technique that produce virulent and stable fungal propagules (Kassa et al. 2008). Different methods that have been developed produce different type of spores, namely aerial conidia, blastospores, and submerged conidia (Feng et al. 1994). For instance, solid substrate fermentation (SSF) produce aerial conidia, the

primary infective bodies of hyproceals, whereas submerged liquid fermentation (SLF) produce blastopores, or submerged conidia (Jaronski, 2014). The former has been used more commonly since it's productive, cheaper, requires less energy and water, and less prone to contamination (Pham et al. 2010). Another important consideration is that conidia has been reported as the most efficient propagule for field use due to their high infectivity and stability post-application and at dry conditions (Ibrahim et al. 2015). On the other hand, blastospores are hydrophilic and upon storage may lose their viability fast (Pham et al. 2010). Nonetheless, the type of selected process still depends on the desired application and end product, fungal strain, environment, and target pest (Feng et al. 1994).

#### 2.7.1.5.1. Solid-state fermentation

Approved *B. bassiana* based products contain aerial conidia, produced by solid-state fermentation (Lohse et al. 2014). The process uses a natural or an inert solid substrate as a nutrient source and support (Krishna et al. 2005). Various organic materials have been evaluated and used as solid substrate media for mass production including rice, barley, wheat, sugarcane, maize, and millet. Other inorganic materials have been identified such as diatomite and clay granules (Jaronski et al. 2014). SSF takes place through a single or two stage process. A single stage production involves the direct inoculation of the substrate with conidia from a solid culture. In a two-stage production an inoculum is prepared using submerged liquid fermentation and then used for the solid phase (Mascarin and Jaronski, 2016).

*Beauveria bassiana* was produced using a wide range of substrates including wheat bran, rice, burghul, sugarcane (Jaronski, 2014). Pham et al. (2010) obtained a high yield of *B. bassiana* conidial concentration on different grains following a simple and productive procedure. Grains were soaked in distilled water, placed in polyethylene bags and autoclaved at 121 °C for 25 mins. Upon cooling, the bags were inoculated by a previously prepared conidial suspension ( $10^7$  conidia/g) from a stock solution maintained on Potato dextrose agar (PDA) and incubated at 25°C for 14 days. The highest conidial yield of  $1.82 \times 10^9$  conidia/g was obtained on rice. Likewise, the most common used entomopathogens in pest control; *Metarhizium* spp. and *Beauveria* spp. have been mass produced on steamed rice at a commercial scale in Brazil (Alves and Pereira, 1989). Both entomopathogenic fungi, isolated from Lebanese soils, showed high efficiency against arthropod pests such as aphids, spider mites and whiteflies when mass-produced on rice and burghul. In fact, Ibrahim et al. (2015) found that burghul substrate was superior to rice, wheat and vegetable peel in the mass production of aerial conidia of *M. anisopliae* (LIM 1) in terms of yield. Following mass production, conidial yield can be formulated differently depending on the application.

#### 2.7.1.6. Formulation

The formulation process is a critical step in the development of mycopesticides, as it provides the product with commercial “robustness” (Mascarin and Jaronski, 2016). It is performed based on a criteria of enhancing fungal viability and virulence as well as environmental and field persistence; offering safe and easy to use products; and extending storage and shelf-life without altering insecticidal properties (Feng et al. 1994; Mascarin and Jaronski, 2016). A broad range of formulation forms and additive materials have been

developed. Generally, formulations can be dry (granular and powder) as they are mixed with a solid carrier or liquid (oil and water-based formulations), suspended in an aqueous fluid. In pest control, entomopathogenic fungus are mostly formulated as oil dispersions and wettable powders (Wraight et al. 2016).

#### 2.7.1.6.1. Wettable powders (WPs)

Fungal spores are highly hydrophobic, thus hard to suspend in water (Behle et al. 2006). The addition of wetting agents have shown to solve this problem by improving conidial wettability, dispersion and adhesion (Santos et al. 2012). In fact, WPs has become the most favored among developers due to its easy handling, superior physical properties and light weight (Wraight et al. 2016). WP formulations often combine aerial conidia with minerals such as clays and talc that are pulverized into fine, sprayable powders. Other ingredients are added such as surfactants, dispersing agents and inert material (Melo et al. 2020). The powder is mixed with water and applied to plant foliage. Several wettable *B. bassiana* formulations showed high efficacy against arthropod pests such as melon aphid, *Aphis gossypii*, and onion thrips, *Thrips tabaci* (Ezzati-Tabrizi et al. 2009; Wraight et al. 2016).

#### 2.7.1.6.2. Oil dispersions (OPs)

Under field conditions, environmental stress such as high temperatures, intense UV radiation and low humidity can significantly lower the infectivity of entomopathogens (Awan et al. 2021). Since oil and oil-emulsifiers provided protection to fungal spores against these limitations, their use in emulsified suspensions for pest

control increased rapidly (Wraight et al. 2016). Oil-based formulations are usually composed of different combinations of plant-origin oils (e.g corn, soybean, sunflower, and sesame oil), oil-soluble emulsifiers (Tween 20) and water-soluble emulsifiers (Batta et al. 2016). It's important to set equal ratios of oil and aqueous phases to maintain the product stability and ensure high conidial germination and penetration into the host (Batta et al. 2016). An enhanced fungal virulence was observed upon the addition of oils due to their lipophilic nature which allow them to adhere strongly to hydrophobic surfaces of conidial spores and insect cuticle. Also, it was shown that oils or oil-emulsifiers build films on the pest cuticle that provide a suitable microclimate for fungal germination and infection (Malsam et al. 2002). Therefore, a faster speed of kill is expected in oil-based fungal formulations and was reported in arthropod pests under lab conditions (Malsam et al. 2002; Batta et al., 2016). High mortality rates among whitefly nymphs (*Bemisia tabaci*) and aphid adults (*Myzus persicae*) was recorded following the application of *B. bassiana* oil formulation (Olson and Oetting, 1999; Dun et al. 2003). Also, it was demonstrated that adding corn oil to conidial spores of *B. bassiana* improved their thermotolerance and increased germination rates by 70% (Kim et al. 2010; Baroudy, 2018).

#### 2.7.1.7. Compatibility of *B. bassiana* with chemical pesticides

The success of entomopathogenic fungi as biological control agents (BCAs) in IPM systems is highly dependent on their compatibility with chemical insecticides (Rajanikanth et al., 2010). Hypothetically, the combined application of both, whether in rotation or mixed, is suggested to minimize the evolution of pest resistance and outbreaks. While some chemicals such as chlorpyrifos, flufenoxuron, indoxarb,

cypermethrin and fenvalarate were reported incompatible with *B. bassiana*, Imidacloprid showed a positive interaction with multiple strains (Neves et al. 2001; Purwar et al. 2005; Rajanikanth et al. 2010; Abidin et al. 2017; Dannon et al. 2020). In fact, highest mortality rates of onion thrips were recorded upon applying *B. bassiana* and Imidacloprid together under laboratory and greenhouse conditions. Similarly, the control of 3 different populations of *B. tabaci* exceeded 80% when sprayed in alternations of *B. bassiana* with pyrufluquinazon or cyantraniliprole. In the same experiment, mortality rates of nymphs and adults increased significantly when *B. bassiana* was mixed with flonicamid that showed no insecticidal effect on either stages when applied alone. Furthermore, a synergistic effect persisted upon combining *B. bassiana* with lepimectin, milbemectin, abamectin or pyrufluquinazon against *B. tabaci* nymphs (Wari el al. 2020).

#### 2.7.1.8. Compatibility of *B. bassiana* with *A. swirskii*

Although the phytoseiid mite *A. swirskii* has been solely introduced to control thrips and whiteflies, it is often used in a preventative manner to limit pest establishments and control light/moderate populations. At high infestation rates, incorporating compatible BCAs with *A. swirskii* for curative treatments is required. In this context, *B. bassiana* represent a potential candidate since it has no detrimental effects on phytoseiid mites (Numa vergel et al., 2011; Chandler et al. 2005). Both biocontrol agents are known to control whiteflies and thrips without targeting the same life stages, thus eliminating direct competition. On whiteflies, *A. swirskii* feeds mainly on eggs and 1<sup>st</sup> instar nymphs while *B. bassiana* is highly virulent against the 2<sup>nd</sup> and 3<sup>rd</sup>



instars. Similarly, *A. swirskii* attacks immature stages of thrips whereas, adults are the most susceptible to *B. bassiana*. The high capacity of *A. swirskii* to carry and disseminate *B. bassiana* spores, resulted in higher mortality rates of *Diaphorina citri* and *F. occidentalis* compared to *B. bassiana* sprayed alone (Zhang et al. 2015; Lin et al. 2019). These findings suggest that the activity of *A. swirskii* can be twofold; pest control by direct predation and through the transmission of *B. bassiana* spores. Under laboratory conditions, *A. swirskii* protonymphs showed high tolerance to *B. bassiana*. Seidy et al. (2015) reported that susceptibility of *A. swirskii* to *B. bassiana* depends on the fungal strain, application rate and timing.

### **2.8.1. The microbial biocontrol agent: *Bacillus thuringiensis***

*Bacillus thuringiensis* (Bt) is a spore-forming, Gram positive, rod shaped and aerobic bacterium most commonly found in soils and dead insects (Zhang et al. 2016). During sporulation, they are known to produce host-specific insecticidal crystal proteins (Cry), often called  $\delta$ -endotoxins (Moustafa et al. 2018). The specificity of these proteins is particularly important as they are safe to non-target organisms such as predators. Upon the host ingestion. Cry proteins are solubilized in the mid gut of the insect which is then expected to die within 6 to 12 hours (Jouzani et al., 2017). High toxicity was reported among more than 3000 insects belonging to Lepidoptera, Coleoptera, Hymenoptera, Diptera and other orders (Zhang et al. 2016). Among Lepidopterans, the tomato pinworm *T. absoluta* showed high susceptibility to *B. thuringiensis*. Several studies reported high mortality rates among all Bt-treated larval instars of *T. absoluta* (Hernandez et al. 2011; Gowtham et al. 2018; Sandeep Kumar et al. 2020). In addition,

*B. thuringiensis* was shown to reduce the population of *Lirimyza trifolii* (Dipter: Agromyzidae) on beans compared to untreated control, resulting in highest yields. Hence, Bt-based products comprise 90% of microbial based biopesticides currently available in the market that have been extensively in IPM systems (Sandeep Kumar et al. 2020). Commercial Bt-products are available in different formulations such as Powder, Granule, wettable powder, oil dispersion and capsule suspension. The selection of a proper formulation is critical to avoid the loss of insecticidal activity due to UV light and unfavorable temperatures (Fernández-Chapa et al., 2019).

## CHAPTER 3

### MATERIALS AND METHODS

#### **3.1. Field Evaluation of the efficacy of the local fungus *Beauveria pseudobassiana* predatory mites *Amblyseius swirskii* and *Phytoseiulus persimilis* for the management of greenhouse tomato and cucumber pests**

##### ***3.1.1. Field location***

Two large scale commercial size greenhouse trials were conducted in Rmeileh, South Lebanon, at an altitude of 80m above sea level (Fig. 1). Each trial was conducted in two different seasons, the first trial during September-December 2019 on cucumber, while the second between January-April 2020 on tomato. The experimental design was the same in both experiments. Three greenhouses with an area of 325 m<sup>2</sup> (7x46.5m) per greenhouse were allocated for each experiment. The greenhouses were of the traditional arched tunnel type. In each greenhouse, 8 rows were cultivated with approximately 900 to 1000 cucumber plants in the first trial and tomato plants in the second trial. One greenhouse was used as control where conventional agriculture practices were adopted by the farmer; while the other two greenhouses were subject to IPM control measures. The first IPM greenhouse (IPM-A) was set to be primarily controlled by *B. pseudobassiana*, while the second greenhouse (IPM-B) by predatory mites *Phytoseiulus persimilis* and *Amblyseius swirskii*.

A drip irrigation system was used in both experiments to ensure adequate irrigation of crops. In the first experiment, cucumber seedlings, variety 'Saifi', were transplanted in the field on the 11<sup>th</sup> of September 2019 at a rate of 2 plants /m<sup>2</sup>. In the second field experiment, tomato seedlings, variety 'Red Flora' were transplanted at the same rate of 2 plants /m<sup>2</sup> on the 18<sup>th</sup> of January 2020.



Figure 1. Experimental site in Rmeileh area (Yellow border).

### ***3.1.2. Pre-transplanting measures in IPM greenhouses***

The soil was solarized and all greenhouses were equipped with clear plastic polyethylene covers and insect-proof nets that covered the vents. Weeds and plant debris from previous seasons were completely removed by hand without any herbicide application. To remove any recently introduced or remaining whiteflies and thrips, yellow sticky traps (YSCs) and blue sticky traps (BSCs) from the ministry of agriculture extension services were placed at a rate of 1 trap/16m<sup>2</sup> and regularly replaced upon need (Fig. 2). Trap plants, blossomed marigolds (Ferry-morse®) and beans, were grown freely with no insecticides in a greenhouse at the American University of Beirut (AUB) and planted a week before transplanting at a rate of 1 plant/ 32 m<sup>2</sup> (Fig. 3). As some pests were attracted and trapped, the plants were removed using a nylon bag one day before transplanting and replaced by new ones. Data on daily temperature and humidity at an

interval of 15 minutes were recorded by Data loggers (Ebro ®). In the tomato experiment, double sliding doors were constructed to minimize pests' entry.



Figure 2. Pre-transplanting measures in IPM greenhouses.



Figure 3. Trap plants; flowering marigolds

### ***3.1.3. Pre-transplanting measures in the control greenhouse***

In the control greenhouse, the same pre-transplanting procedures were followed excluding the introduction of trap plants, yellow/blue sticky traps and double sliding doors. In the tomato experiment, soil fumigation was applied to greenhouses before the beginning of the growing season.

### ***3.1.4. Post-transplanting measures***

#### **3.1.4.1. Control greenhouse: pesticide sprays**

In the first trial, the farmer spraying program included at least 6 pesticide sprays during the growing season, each consisting of a mixture 2 pesticide active ingredients, applied 1-2 weeks intervals. The applied insecticides/acaricides targeted mites, thrips, whiteflies and aphids. Details of pesticides applied in the control greenhouse, including dates of application and active ingredients are provided in Appendix II, Table 1.

#### **3.1.4.2. Pests and predators scouting**

In both experiments, the plants were left open under natural infestation of pests. Insect/mite and predator scouting was done on a weekly basis in all greenhouses. Five randomly selected cucumber/tomato plants were inspected per row, summing up a total of 40 plants per greenhouse. In the trial, three leaves from the upper, middle and lower parts were scouted, that is 120 leaves per greenhouse. Populations of adult and immature stages of arthropod pests; whiteflies, spider mites, thrips and aphids were recorded and the average of each was calculated. Whereas in the second trial, three compound tomato leaves were scouted for *Tuta absoluta* and leafminer population from the upper, middle and lower parts. The same monitoring framework was performed for *P. persimilis* and *A. swirskii*. During the growing season in IPM greenhouses, sticky traps were replaced by new ones upon need and highly infested trap plants were removed and replaced by newly grown ones.

### 3.1.4.3. Application of *B. pseudobassiana*

#### 3.1.4.3.1 Fungal isolate

A local strain of *B. pseudobassiana* originally isolated from a cedar web-spinning sawfly in North Lebanon was used in all experiments. Fungal spores obtained from long-term stored fungal cultures with 10% glycerol incubated at -80°C were transferred under sterile conditions by a plastic loop to a 9 cm petri-dish Potato Dextrose Agar (PDA) (HiMedia<sup>®</sup>) and incubated at 25°C and a photoperiod of 16 hours light and 8 hours dark. Pure cultures were maintained by repeated sub-culturing on PDA plates every 14 days.

#### 3.1.4.3.2. Preparation of conidial suspensions

Following incubation, aerial conidia from a two-week old *Beauveria* culture grown on PDA plates were suspended in 10 mL sterile distilled water and 0.01% Tween-20 (Sigma<sup>®</sup>) acting as a wetting dispersing agent. Fungal spores were collected by gently scrapping the culture using a sterile plastic loop. The obtained suspension was filtered through three layers of sterilized cheesecloth to remove any remaining hyphal fragments and poured in a 50 ml falcon tube containing distilled water. The resulting suspension was used as stock solution. Conidial counts were performed using an improved Neubauer haemocytometer and diluted to a concentration of  $10^7$  conidia mL<sup>-1</sup> to be used as conidial inoculum.

#### 3.1.4.3.3. Preparation of large amounts of inoculum

One kg of burghul and 500 ml of distilled water (dH<sub>2</sub>O) were placed in autoclavable polypropylene bags (30 x 60 cm), shaken well to obtain a homogenous mixture, sealed using Food Saver Vacuum Sealer<sup>®</sup> and autoclaved at 121°C, 15 psi for 16 minutes. After

cooling, each bag was disinfected by 70% alcohol and a sterile syringe containing 30 ml of conidial inoculum ( $10^7$  conidia  $\text{mL}^{-1}$ ) was carefully injected into each bag. The puncture was resealed by a plastic tape right after the needle withdrawal. Inoculated bags were gently shaken to ensure proper distribution of the inoculum and placed in a controlled environment at  $25 \pm 2^\circ\text{C}$  and 70% relative humidity for 2 weeks until fully colonized.

#### 3.1.4.3.4. Spraying formulation

Oil-based fungal formulations were prepared on the same day of application. After 2 weeks of incubation, 1kg of burghul was soaked in 1-1.5L of water for 30 mins. The solution was then filtered using a sieve and 5 layers of cheesecloth to remove impurities to obtain a conidial suspension of  $10^9$  conidia  $\text{g}^{-1}$ . The spraying formulation was prepared by diluting the conidial suspension to a concentration of  $10^8$  conidia  $\text{mL}^{-1}$  and amending it with 0.01% Tween 20 and 1% corn oil.

#### 3.1.4.3.5. Field application

The fungal formulation was sprayed depending on pest scouting results with different volumes based on the infestation level. In the first experiment, in IPM-A, five sprays of *Beauveria* conidial suspensions were carried out to control all pests. At the beginning of the season, 30L of solution were sprayed on the plants to control aphids. The volume increased to 70L with the growth of the plants. In IPM-B, during the middle of the season, *Beauveria* conidial suspensions were sprayed against aphids in hot spot treatments. A uniform spray coverage of plants was achieved using a 20L battery operated knapsack sprayer (Montana<sup>®</sup>). In the second experiment, five sprays of *Beauveria* conidial



suspensions were performed in IPM-A to control pests. An additional spray of *B. thuringiensis* was done between the last two spray of *Beauveria* to eliminate any risk of phytotoxicity. Similarly, five sprays of *B. thuringiensis* were carried out in IPM-B.

#### 3.1.4.4. Release of predatory mites *A. swirskii* and *P. persimilis*

##### 3.1.4.4.1. Mass production of *A. swirskii* and *P. persimilis*

The local strain of *A. swirskii* that was originally collected from the Batroun area and identified previously by morphological and molecular means, was obtained from the Plant Pathology Laboratory at AUB. The mite was reared in plastic boxes containing a culture media of wheat bran and wheat mite *Carpoglyphus lactis* (Linnaeus). Rearing boxes were incubated at 25°C and 60-70% RH.

The local strain of *P. persimilis* obtained from South Lebanon and identified previously by morphological and molecular means was supplied by the Plant Pathology Laboratory at AUB as well. The predator was reared and maintained on glasshouse bean plants previously infested with *T. urticae* as prey food. Regular introductions of new bean plants and spider mites were performed to maintain a continuous supply of *P. persimilis*. Rearing took place at 25°C and 60-70% RH.

##### 3.1.4.4.2. Introductions of *A. swirskii* and *P. persimilis*

The standard biological control-based IPM strategy for cucumber was based on the release of *A. swirskii* for the control of *B. tabaci* and *F. occidentalis* and *P. persimilis* for the control of *T. urticae*. The timing and release rate of natural enemies was decided on according to the scouting results of pest populations and crop stage. During the first experiment in IPM greenhouse (B), four introductions of *A. swirskii* were carried out at a

release rate of 25 mites/ m<sup>2</sup> in the beginning of the season and increased with the growth of cucumber plants to 50 mites/m<sup>2</sup>. Three releases of *P. persimilis* were performed in hotspots due to the non-uniform spread of *T. urticae* populations.

In the second experiment, *T. absoluta* was the dominant pest controlled primarily by *Bacillus thuringiensis*. The absence of *T. urticae* during the experimental season eliminated the need for any introductions of *P. persimilis* and *A. swirskii*.

#### 3.1.4.5. Application of *B. thuringiensis*

In the second experiment, *Bacillus thuringiensis* Subsp. *kurstaki* (Bt) (JAVELIN WP) (24 x 10<sup>9</sup> spores) was applied in IPM greenhouse (B) to control *T. absoluta*. A spray suspension of 20g of *B. thuringiensis*, 20g of sugar and 200 ml of citric acid (1%) in 20L of water was prepared on the same day of application. A total of five sprays were done throughout the experiment.

#### 3.1.4.6. Pesticides sprays in IPM greenhouses

In the first trial, a single insecticidal spray of Coragen® 20 SC (Chlorantraniliprole) was sprayed against aphids. Throughout the growing season of the second trial none of the two IPM greenhouses received any pesticide spray.

#### **3.1.5. Statistical analysis**

Statistical analysis was performed using IBM SPSS statistics 25. In both experiments, statistical analysis evaluated the control efficiency of the IPM strategy to that of the conventional plant protection strategy by comparing the population of each

pest in the IPM greenhouse to that in the control greenhouse, using one-way ANOVA and univariate analysis.

### **3.2. Assessing the compatibility of *B. pseudobassiana* and *A. swirskii* in the management of whitefly *B. tabaci* in small scale experiments**

#### ***3.2.1. Preparation of plant material***

Cucumber, *Cucumis sativus* 'Beit Alpha', was used as a source of plant material for the experiment. Seeds were sown in 50 cell plug trays and maintained in a glasshouse at 25-28°C, 60-70% RH and 16: 8 (Light: Dark hours) at the American University of Beirut. After two weeks, plants were transplanted in 15 cm diameter pots filled with a mixture of peat moss, coco peat and perlite. Upon reaching three true leaf stages, the plants were transferred to a second glasshouse with the same conditions and placed in insect-proof cages at a rate of 3 cucumber plants per cage.

#### ***3.2.2. Whitefly rearing and collection***

Insects were reared on potted cucumber plants in insect-proof cages (55 cm x 55 cm x 55 cm) in a controlled environment glasshouse at 25-28°C and 60-70% RH. Cucumber plants were irrigated every other day and replaced by other healthy ones when dead to maintain *B. tabaci* populations. Adults of *B. tabaci* were collected, by a cordless vacuum cleaner (Bosch®).

#### ***3.2.3. Fungal material***

Conidial suspensions of the local strain of *B. pseudobassiana* were prepared following the same procedure undertaken for large scale experiments. A conidial formulation of  $10^8$  conidia mL<sup>-1</sup>, 0.01% Tween 20 and 1% corn oil was used.

#### **3.2.4. *Amblyseius swirskii* culture**

The local strain of *A. swirskii* was prepared following the previously described method in section 3.1.4.4.

#### **3.2.5. *Treatments and data collection***

A small scale experiments was conducted in a glasshouse, located in the greenhouse area, Department of agriculture, American University of Beirut. The following treatments were done: (1): *A. swirskii*, (2): *B. pseudobassiana*, (3): *A. swirskii*, +*B. pseudobassiana*, (4): oil and (5): control sprayed with water. All treatments were replicated 3 times (3 cages); a total of 9 cucumber plants/treatment. For each treatment, 15 *B. tabaci* adults were released in an insect proof cage containing 3 cucumber plants during the first week of the experiment and left for 7-10 days to lay eggs. In total, 2 releases of *B. tabaci* were performed throughout the experiment on week 1 and week 4. Ten days after releasing the *B. tabaci* adults, *A. swirskii* was released at a rate of 50 mites per cage and *B. pseudobassiana* was sprayed covering the whole plant in the respective treatments. An additional spray of *B. pseudobassiana* was done in the third week. Average numbers of *B. tabaci* nymphs and *A. swirskii* populations were recorded on a weekly basis over a period of 7 weeks.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### **4.1. Large scale field trials in commercial greenhouses to evaluate the efficacy of the local fungus *Beauveria pseudobassiana*, and local predatory mites *Amblyseius swirskii* and *Phytoseilus persimilis* against cucumber pests within an integrated pest management system**

##### ***4.1.1. Ambient conditions***

The retrieved data on temperature and RH variations in the cucumber trial are presented in Appendix II, figures 13 and 14. Throughout the growing period of the trial which was conducted during the fall season (September to December 2019), the mean daily temperatures varied from 31.281°C on week 2 and 19.08°C on week 10 (minimum and maximum mean temperatures being 15°C and 32°C, respectively). The average relative humidity fluctuated between 51 and 90%.

##### ***4.1.2. Population dynamics of pests/predators in control and IPM greenhouses***

For the following field trial, results of the population dynamics of arthropod pests; whiteflies, thrips, aphids and mites and their corresponding natural enemies in the control and the two IPM greenhouses (IPM-A (*B. pseudobassiana*) and IPM-B (*P. persimilis* and *A. swirskii*), are presented in Figures 4-8.

##### ***4.1.3. Whitefly management***

In the control greenhouse, the farmer performed 6 insecticidal sprays during the growing period using 2 active ingredients per application (Appendix III, Table II). During the first 5 weeks (Sep 25-Oct 23), the average whitefly adult population was

relatively low ( $< 1$  adult/leaf) (Fig. 4). This time corresponds to a period where maximum temperatures were as high as 41 to 54° C; a thermal range that was reported lethal to the development and survival of *B. tabaci* adults and immatures (Xaio et al. 2016). Accordingly, the number of average whitefly nymphs was almost negligible along that period (Fig. 5). By that time, the farmer had already applied 3 insecticidal sprays. On the first week of November, with the drop in average and maximum temperatures, the number of whitefly adults increased and nymphal stages started to emerge. Although this slight increase was accompanied by 3 additional insecticidal sprays, a continuous growth in adult population persisted reaching a peak of 4.12 adult/leaf (Fig. 1), slightly lower than the economic threshold level ETL (5 whiteflies/leaf). Similarly, a consistent and faster multiplication rate of whitefly nymphs was observed, exceeding the ETL by Mid-November, and later growing exponentially to 19.7 nymphs/leaf at the end of the experiment (Fig. 5). The inefficiency of the applied chemicals might be linked to different factors including wrong application methods, rate or timing, insufficient leaf coverage, but mostly to whitefly resistance to abamectin and acetamiprid which have been repeatedly used without alterations.

In IPM-A greenhouse, a total number of five *Beauveria* sprays during the cucumber growing period provided sufficient control against the population of whiteflies and maintained it below the ETL (Appendix III, Table I). The initial number of whitefly adults dropped from 1.23 to 0.35 adult/leaf during the second week, and remained below 1 adult/leaf all way through (Fig. 4). Average density of whitefly nymphs peaked part way through the season (2.57 nymphs/leaf) and declined progressively afterwards by the end of the experiment to a level of 1.06 nymphs/leaf (Fig. 5). Parasitized whitefly nymphs

due to fungal infection were observed in the greenhouse from the 7<sup>th</sup> week onwards. An average of 2 parasitized nymphs/ leaf was recorded during the last 4 weeks.

In IPM-B greenhouse, *A. swirskii* was released to control whitefly populations on cucumber, and succeeded in maintaining the number of adults and immatures below ETL during the season (Appendix III, Table I). To initiate the early establishment of the mite and with the presence of few numbers of whitefly adults, 2 releases of *A. swirskii* at a rate of 25 mites/m<sup>2</sup> were performed on the 1<sup>st</sup> and 3<sup>rd</sup> week of the experiment (Sep 25 and Oct 9). Although maximum recorded temperatures were above 40° C until the third week of October, *A. swirskii* was active and maintained whiteflies at low levels (< 0.5 adult or nymph/leaf) (Appendix II, Fig. II; Fig. 1). Later, average temperatures dropped during the first half of November to a range between 21.5-26.8° C, which favored the development of whiteflies (Appendix II, Fig. I). Consequently, the rise in whitefly nymphs on the 1<sup>st</sup> of November was followed by a release of *A. swirskii* at a rate of 50 mites/m<sup>2</sup>. Thereafter, the ratio of whiteflies: *A. swirskii* reached 4.8 whiteflies available for each mite. Two weeks later, the number of whitefly nymphs was lowered but a slight increase among adults was recorded, so a final release of *A. swirskii* at a rate of 50 mites/m<sup>2</sup> was carried out. By that time, the ratio of whiteflies: *A. swirskii* declined to 2.12 whitefly for each mite and the average whitefly population dropped to a level of 0.53 adult and 1.2 nymphs per leaf by the end of the experiment (Fig. 1&2). The highest peak of *A. swirskii* was 1.45 mite/leaf recorded on December 4.

There was a significant difference in the average number of whitefly adults and nymphs between treatments ( $F_{adults} = 67.998$ ,  $df = 2$ ,  $P < 0.01$ ;  $F_{nymphs} = 39.851$ ,  $df = 2$ ,  $P < 0.01$ ), weeks ( $F_{adults} = 15.770$ ,  $df = 9$ ,  $P < 0.01$ ;  $F_{nymphs} = 19.379$ ,  $df = 9$ ,  $P < 0.01$ ) as well as treatment\*week ( $F_{adults} = 8.222$ ,  $df = 18$ ,  $P < 0.01$ ;  $F_{nymphs} = 11.579$ ,  $df = 18$ ,  $P < 0.01$ ).

0.01). Among whitefly adults, a continuous increase in population was observed in the control starting November till the end of the season. In both IPM greenhouses, the population remained relatively low throughout the experiment with slight fluctuations, and no significant difference between the two greenhouses was observed except for week 2. By comparison, the number of whitefly adults recorded in the control greenhouse at the end of season was; 4.125 adult/leaf. This is compared to an average of 0.64 and 0.53 adults/leaf in IPM-A and IPM-B, respectively. This difference represents a significant relative reduction of 84.5 % in IPM-A and 87.15 % in IPM-B. A similar pattern persisted among the nymphal population but at higher densities compared to adults. The population grew consistently in the control greenhouse all through the experiment, while it was suppressed in the IPM greenhouses. There was no significant difference within any week of the experiment between the two IPM greenhouses. On the last week, the average number of nymphs reached 19.71, 1.06 and 1.2 nymphs/leaf in the control, IPM-A and IPM-B greenhouses, respectively. Relatively, the average population was significantly lower in IPM-A by 94.6 % and IPM-B by 94 % as compared to the pesticide control.

The ability of *A. swirskii* to suppress whiteflies on cucumber under field conditions have been well documented (Messelink et al. 2005; Calvo et al. 2012). Nomikou et al. (2001) reported a reduction in *B. tabaci* by 16 to 21-folds upon the release of *A. swirskii* on cucumber plants compared to the control. The current study has also revealed that *A. swirskii* was able to forage and establish at high temperature; suggesting a thermotolerant nature of the local isolate of *A. swirskii*. Maximum temperatures were as high as 41 to 54°C during the first 5 weeks (Sep 25-Oct 23), and ranged between 40 to 45 °C during the last 2 weeks of November (Appendix II, Fig. I&II). This is particularly important and it is relevant to assess the thermal limit of the local *A. swirskii* isolate since the upper



development threshold of the mite was previously established at 37.4 (Lee and Gillespie, 2011). Nevertheless, careful and precise monitoring of pests remains critical during warm periods and weekly releases at adequate rates (25, 50 mites/m<sup>2</sup> or more) depending on infestation level and plant age is recommended to ensure proper control and avoid any sudden outbreak of pests.

The use of local *B. pseudobassiana* provided sufficient control against *B. tabaci*. In the present study, the application of 5 fungal sprays on greenhouse cucumber resulted in significant lower reductions in *B. tabaci* adult and nymphal populations of 84.5 and 94.6 %, respectively, compared to the control. Overall findings from previous studies on commercial *B. bassiana* strains showed similar levels of control among whiteflies. Under lab conditions, *B. bassiana* was effective against *B. tabaci* (81.1% mortality) compared to 8.8% in the control (Bugti et al. 2018). Abdel-Raheem & Al-Keridis (2017) found that *B. bassiana* was highly toxic against *B. tabaci* adults under field conditions, causing more than 90% mortality rates after the 3<sup>rd</sup> application. Recently, a field experiment under protected conditions was conducted over 2 years to evaluate the pathogenicity of multiple biopesticides against *B. tabaci* on cucumber. The application of 3 sprays of liquid formulation of bio-power (*B. bassiana* at  $1 \times 10^9$  CFU/ml) at 10-day interval caused significant reductions of 89-91.2 % and 89.9-85.8% among nymphs as well as 58.2-62.3% and 61.4-65.4% in adults compared to the control in 2017 and 2018, respectively (Ghongade et al. 2021). In parallel, Prithiva et al. (2017) demonstrated the effectiveness of different formulations of *B. bassiana* isolate against *B. tabaci* on tomato. The study showed high reduction in population over control, with the oil formulation being the most effective.

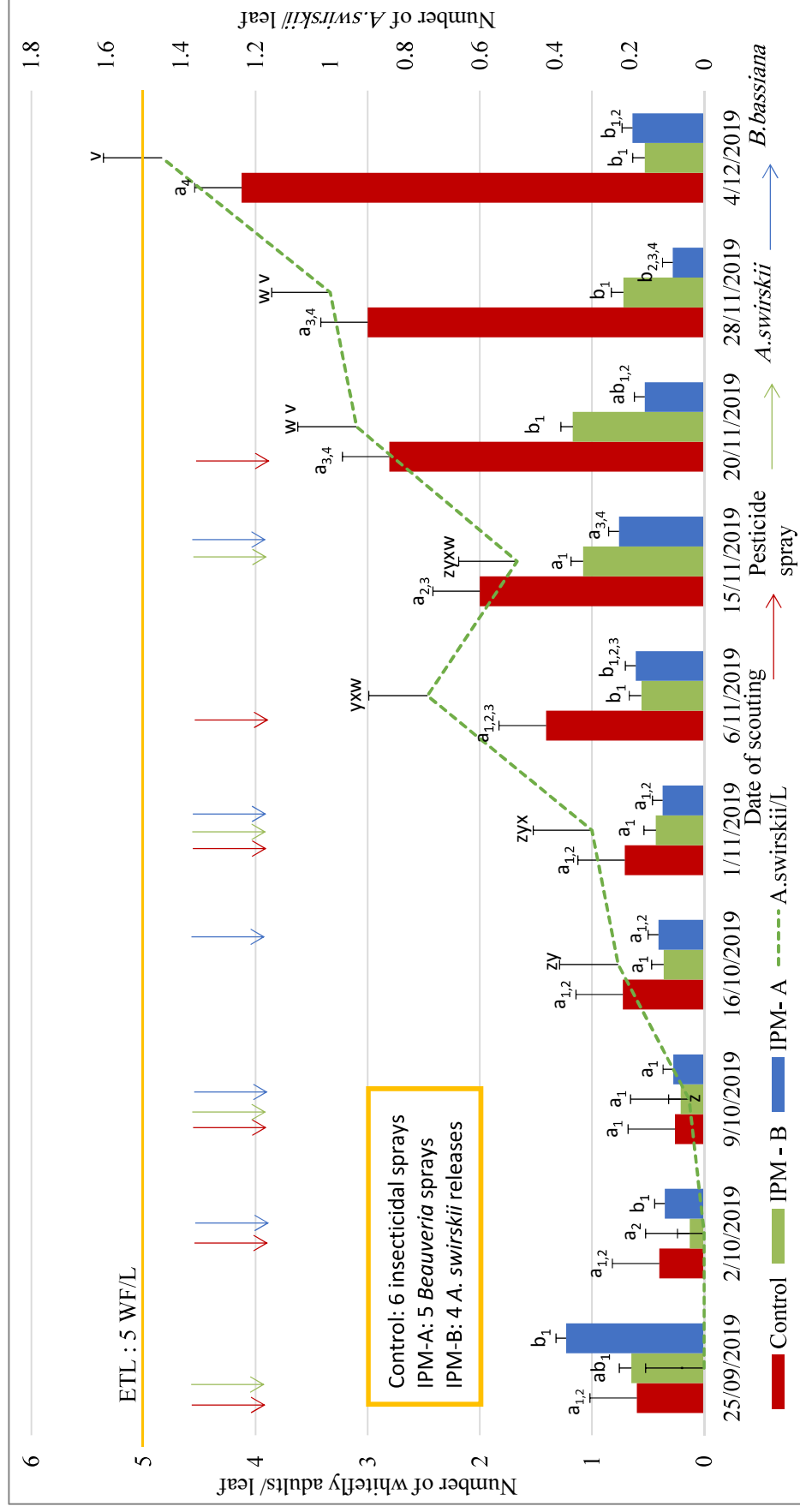


Figure 4. Average whitefly adult and *A. swirskii* populations recorded on cucumber leaves throughout the growing period in the control, IPM-A and IPM-B greenhouses, at Rmeileh. A total of 6 insecticidal/acaricidal sprays, 5 *Beauveria* sprays and 4 releases of *A. swirskii* were performed in control greenhouse, IPM greenhouses A and B, respectively. WF/L: whitefly per Leaf; ETL: Economic threshold level. Different letters indicate statistically different values within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).

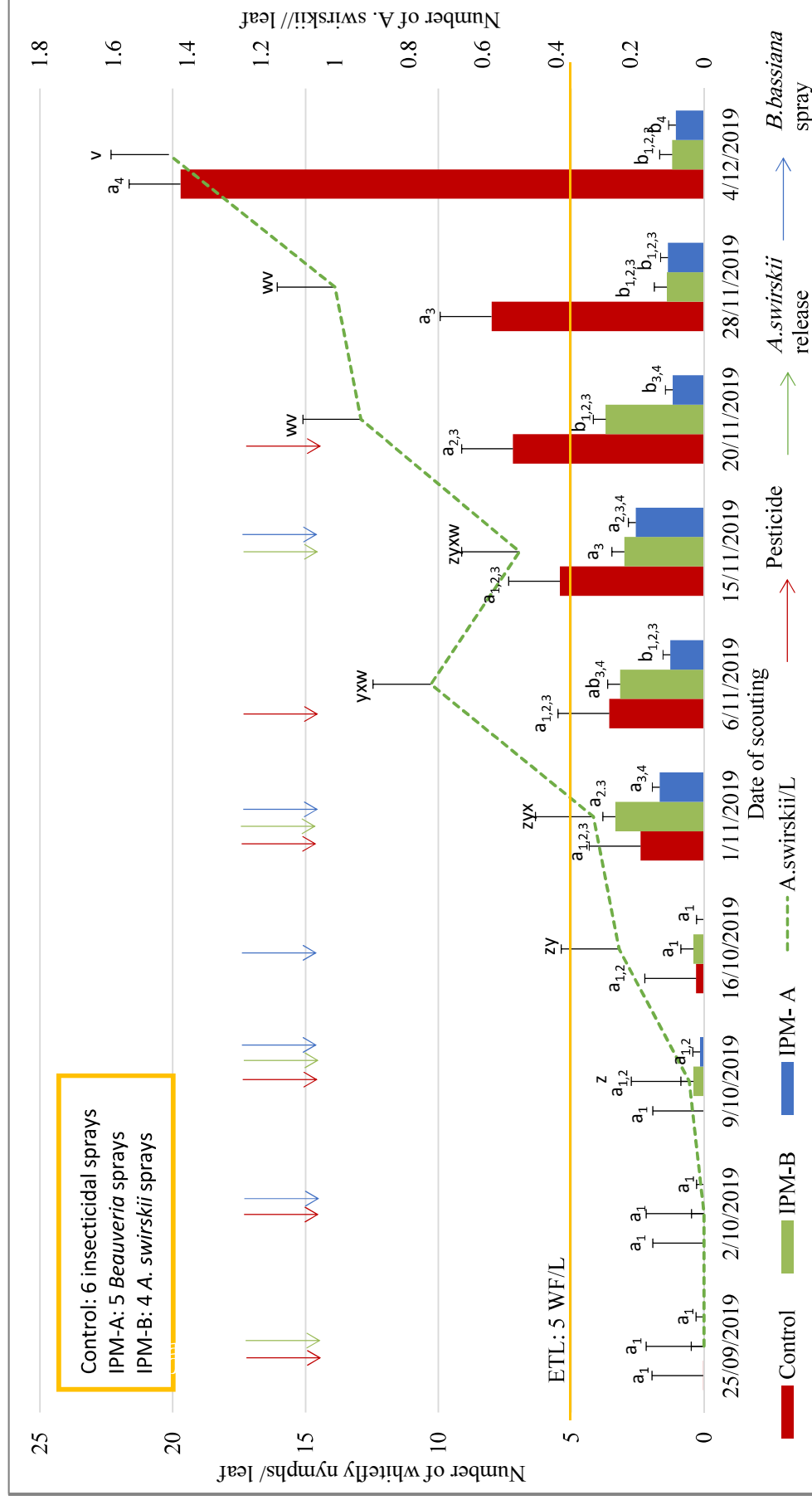


Figure 5. Average whitefly nymphs and *A. swirskii* populations recorded on cucumber leaves throughout the growing period in the control, IPM-A and IPM-B greenhouses, at Rmeileh. A total of 6 insecticidal/acaricidal sprays, 5 *Beauveria* sprays and 4 releases of *A. swirskii* were performed in control greenhouse, IPM greenhouses A and B, respectively. WF/L: whitefly per leaf; ETL: Economic threshold level. Different letters indicate statistically different values within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).

#### 4.1.4. Thrips management

In the control greenhouse, thrips were almost absent throughout the growing season (Fig. 7). The applied insecticides were able to suppress thrips population. During the experiment. The sprayed active ingredients were: Abamectin, Acetamiprid, and Lambda-Cyhalothrin.

In IPM-A greenhouse, the thrips population was almost negligible as well. The application of five *Beauveria* sprays during the cucumber season maintained the average number of thrips below the ETL level of 1.3 thrips/leaf (Appendix III, Table I; Fig. 7).

In IPM-B greenhouse, *A. swirskii* provided sufficient control against thrips population and maintained it below the ETL throughout the growing period (Appendix III, Table I). An emergence of thrips was observed during the 1st and 2<sup>nd</sup> week of the season, however, the population was suppressed following two introductions of *A. swirskii* of 25 mites/m<sup>2</sup>. During the first half of November, two additional releases of *A. swirskii* at a rate of 50 mites/m<sup>2</sup> were performed to control both whiteflies and thrips as favorable temperatures for the pests' development persisted. After the last release of *A. swirskii*, the mite population doubled within 2 weeks from 0.5 to 1 mite/leaf on Nov 28 (Fig. 4). During that time, a slight increase in the number of thrips was observed from 0.2 to 0.3 thrips/leaf by the last week of November. On Nov 28, the ratio of thrips: *A. swirskii* was 3.33 thrips available for each mite. At this ratio, a drop was recorded among thrips population by the following week on Dec 4 to reach 0.2 thrips/leaf. In parallel, the highest peak of *A. swirskii* was recorded by that time; 1.45 mite/leaf (Fig. 7).

There was a significant difference in the average number of thrips between treatments ( $F = 21.001$ ,  $df = 2$ ,  $P < 0.01$ ), weeks ( $F = 7.059$ ,  $df = 9$ ,  $P < 0.01$ ) as well as treatment\*week ( $F = 7.034$ ,  $df = 18$ ,  $P < 0.01$ ). Throughout the season, the thrips population was suppressed and maintained below ETL in all the three greenhouses. The use of *Beauveria* was almost statistically equivalent to chemical insecticides in reducing thrips population. As for *A. swirskii*, the population was slightly higher than the latter two yet below the ETL.

The efficacy of *A. swirskii* against *F. occidentalis* has been reported on cucumber and sweet pepper under laboratory and greenhouse conditions (Belda and Calvo, 2006; Messelink et al. 2008, Nomikou et al. 2010). We evaluated the potential of *A. swirskii* in the simultaneous control of whiteflies and thrips on greenhouse cucumber plants, compared to the efficacy of chemical control. A total number of four *A. swirskii* releases was sufficient to maintain whitefly and thrips populations below the economic threshold of 5 whiteflies/leaf (Shen et al. 2005) and 1.3 thrips/ leaf (Steiner, 1990) without the use of chemical products. Whereas in the control greenhouse, the application of 6 insecticide sprays maintained thrips population below ETL, but failed to do so against whiteflies leading to heavy infestations. Although the control efficiency of *A. swirskii* against *B. tabaci* and *F. occidentalis* either separately or simultaneously had been reported on greenhouse cucumbers (Calvo et al. 2005; Messelink et al. 2008); our results further indicate a greater control of the mite compared to widely used chemical insecticides.

Local *B. pseudobassiana* was able to suppress *F. occidentalis* population growth showing an equivalent control activity to chemical pesticides in the control greenhouse. Our results are similar to those of Lee et al. (2017) who recorded 75 and 90% population

reductions among *F. occidentalis* 20 and 40 days post *Beauveria* application under glasshouse conditions (chemical control: 85% reduction). In the field trial on cucumbers, the fungal isolate showed 90% control efficacy, similar to that of spinetorm, a semi-synthetic insecticide (85% efficacy). In vitro, Gao et al. (2012) evaluated aqueous suspensions (0.05% Tween-80 in sterile H<sub>2</sub>O) of five *Beauveria* strains for control of western flower thrips. Strain RSB was the most virulent, causing 96% mortality at  $1 \times 10^7$  conidia mL<sup>-1</sup>. In greenhouse trials, significant reductions in adult and larval populations were observed on broccoli even though greenhouse temperatures reached 38°C. Similarly, our results showed that the oil-based formulation of *B. pseudobassiana* was effective as a foliar application against *F. occidentalis* as well as *B. tabaci*, *T. urticae* and *M. persicae* at elevated temperatures greater than 40°C along most of the growing season. In fact, emulsifiable oil-based formulations were reported to protect fungal conidia from the adverse effects of high temperatures (De Oliveira et al. 2

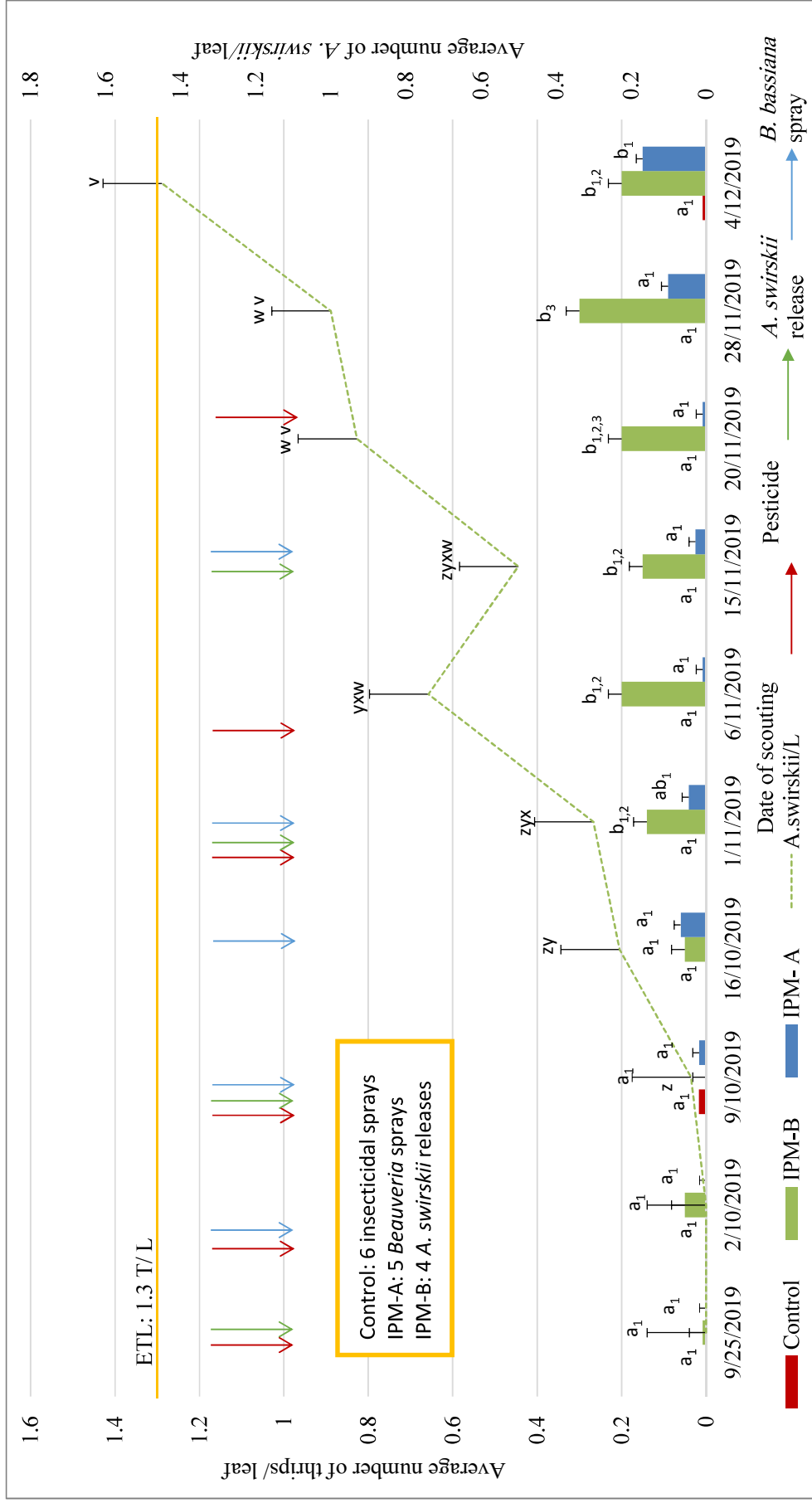


Figure 6. Average thrips and *A. swirskii* populations recorded on cucumber leaves throughout the growing period in the control, IPM-A and IPM-B greenhouses, at Rmeileh. A total of 6 insecticidal/acaricidal sprays, 5 *Beauveria* sprays and 4 releases of *A. swirskii* were performed in control greenhouse, IPM greenhouses A and B, respectively. WF/L: whitefly per Leaf; ETL: Economic threshold level. Different letters indicate statistically different values within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).

#### 4.1.4. Two-spotted spider mite management

In the control greenhouse, the spider mite population was maintained below the economic threshold level of 2 mites/ leaf till the beginning of November. Thereafter, the average number of spider mites reached 3.358 mites/leaf, and fluctuated moderately above the ETL until Nov 20. Although the farmer applied 3 insecticidal sprays during that period including 3 active ingredients; abamectin, acetamiprid, and lambda-Cyhalothrin, the population increased rapidly by 4.6 folds to an average of 19.72 spider mite/leaf at the end of November. On the last week, an outbreak in population took place reaching a level of 40.17 spider mite/leaf (Fig. 6).

In IPM-A greenhouse, five *Beauveria* sprays during the cucumber growing season suppressed the population of spider mites (Appendix III, Table I). The average number remained below 1 spider mite/leaf throughout the experiment except for the period during Mid October where 2 peaks were recorded; 1.819 and 1.809 spider mite/leaf (Fig. 6).

In IPM-B greenhouse, *P. persimilis* was released to control the spider mite population on cucumbers (Appendix III, Table I). During the first two weeks of growing season, the number of spider mites was almost null. The first symptoms of infestation were observed during Mid October within a small limited area in the greenhouse, so two hot spot treatments of *Phytoseiulus* were applied at a rate of 12 mites/m<sup>2</sup>. On the last week of October, we were not able to reach the experimental site due to blocked roads by protesters and thus were not able to perform any release of predatory mites. The week after on Nov 1, *Phytoseiulus* started to establish in the greenhouse but at a lower rate than the population of spider mites which have exceeded the ETL by then; 5.7 mite/leaf. Therefore, an additional hotspot treatment was done



at the same rate as before but covering a wider greenhouse area of 80 m<sup>2</sup>. The sufficient number of spider mites allowed for the proper establishment of *Phytoseiulus*, which reached a peak of 2.5 *Phytoseiulus*/leaf and corresponding to a ratio 1.76 spider mites available for each *Phytoseiulus*. Consequently, a gradual decrease in the number of spider mite was observed, falling below the ETL after 3 weeks of the last *Phytoseiulus* release to an average of 0.26 spider mite/leaf (Fig. 6). At this point, the ratio of spider mites available for each *Phytoseiulus* was 0.136. By the end of the season, the number of spider mites was negligible and the population of *Phytoseiulus* dropped at once in the absence of prey as food source.

A significant effect was observed between treatments (treatments ( $F = 22.127$ ,  $df = 2$ ,  $P < 0.01$ ), weeks ( $F = 5.245$ ,  $df = 9$ ,  $P < 0.01$ ) as well as treatment\*week ( $F = 6.657$ ,  $df = 18$ ,  $P < 0.01$ ) among spider mite population. This proves that the biocontrol agents *P. persimilis* and *B. pseudobassiana* were able to control spider mites effectively and prevent a pest outbreak, unlike the chemical pesticides. The population of TSSM grew above ETL starting from the 2nd of November and remained so throughout the season where it peaked to 40.17 at the end. However, it was maintained below ETL in IPM-A all through the season. In IPM-B, the population peaked above ETL on the 1<sup>st</sup> of November but declined progressively reaching null at the end of the trial. By comparison, the greatest number of spider mites was recorded on the last week in the control greenhouse; 40.17 mite/leaf. This is compared to an average of 0.05 and 0 spider mites per leaf in IPM-A and IPM-B, respectively. This difference represents a relative reduction of 99.8 % in IPM-A and 100 % in IPM-B.

Our findings confirmed a high potential of *P. persimilis* in controlling two-spotted spider mite populations on cucumber plants. While the number of TSSM in the control exceeded

ETL starting the first of November till the end of the season where it peaked to 40.17 mite/leaf, the population in the IPM greenhouse remained below the ETL most of the season, thus recording significantly higher control level. The predation activity of *P. persimilis* has been widely documented in open field and greenhouse crops (Opit et al. 2004; Ullah and Lim, 2017; Yanar et al. 2019). Successful control, however, is dependent on sufficient release rates of the mite and proper timing given that the biotic conditions in protected cultivation favor the development of *T. urticae* (Khalequzzaman et al. 2007; Stravinides et al. 2010). In the current study, the release of *P. persimilis* at a rate of 12 mite/m<sup>2</sup> right after first appearance of TSSM infestations was critical for sufficient control. In parallel, a correct balance between predator and prey should be maintained. Following the two releases of the mite, the ratio of *P. persimilis*: spider mites was 1:4. Thereafter, the population of *P. persimilis* grew to reach its highest peak corresponding to a ratio of 2 spider mites for each *P. persimilis*. Then, the number of *P. persimilis* started to decline with the decrease in spider mite population. This implies that the optimal ratio of *P. persimilis*: spider mites on greenhouse cucumbers must be maintained at or below 1:4. In agreement with this, Opit et al. (2004) found that keeping a consistent ratio of 1:4. *P. persimilis* to spider mites significantly reduced the populations of *T. urticae* on greenhouse ivy geraniums. Moreover, the mite was able to forage and establish at high temperatures; maximum temperatures after the 1<sup>st</sup> release of *P. persimilis* and during ranged between 31.7 and 49.98°C.

A further insight of this study is the indication that the simultaneous introduction of *A. swirskii* for the control of whitefly and thrips along with *P. persimilis* for the control of spider mites is possible. Previous studies found that the introduction of multiple phytoseiid mites

at once can mediate trophic interactions; competition for the same food source, inter and intra-guild predation when the density of preferred prey is low (Albendín et al. 2015). In the absence of alternative and preferred food source, intraguild predation by *A. swirskii* mites on *P. persimilis* was observed (Maleknia et al. 2016). However, an effective pest control was achieved when the two mites were released in combination each for a specific target; *A. swirskii* against *F. occidentalis* and *P. persimilis* against *T. urticae* (Lanzoni et al. 2017). The current work showed that introducing *A. swirskii* and *P. persimilis* based on the availability of their preferred food allowed for their simultaneous use. This is supported by the fact that during the growing period from Oct 16 to Nov 6, both population of *P. persimilis* and *A. swirskii* grew concurrently.

The local strain of *B. pseudobassiana* showed high virulence against *T. urticae*. Our results showed a 99.8% reduction in *T. urticae* populations compared to the control on cucumber under commercial greenhouse conditions. The efficiency of different *Beauveria bassiana* isolates ( $1 \times 10^8$  spore/ml) against TSSM was reported under laboratory and small scale experiments (Negach et al. 2014; Shin et al. 2017; Yucel et al. 2021). Field efficacy of *Beauveria* was evaluated in greenhouse and field trials. On commercial greenhouse peppers, the application of *B. bassiana*-based product (Naturalis,  $2,3 \times 10^7$ ) twice at 5-day intervals showed high efficacy (>97%) in *T. urticae* control. In the current study, a 70% reduction in *T. urticae* population was observed after applying three *B. pseudobassiana* sprays at weekly interval. It must be noted that during the period from the first to the third treatment, mean temperatures ranged between 27-32.5°C and maximum temperature were above 40°C. The strain B. R444 of *B. bassiana*, applied at rate of  $4.2 \times 10^6$  conidia/ml, caused 60-86% mortality

of adult mites 7 days after treatments under greenhouse conditions on cucumber and eggplant. Under field conditions, the fungal strain suppressed spider mite populations when applied at  $1.6 \times 10^8$  conidia/ha at 1 or 2-week intervals (Gatarayiha et al. 2010). Short spraying intervals of 1 or 2 weeks were found the most efficient when controlling two-spotted spider mite since they have a life cycle between 7 and 14 days (Meyer, 1995)

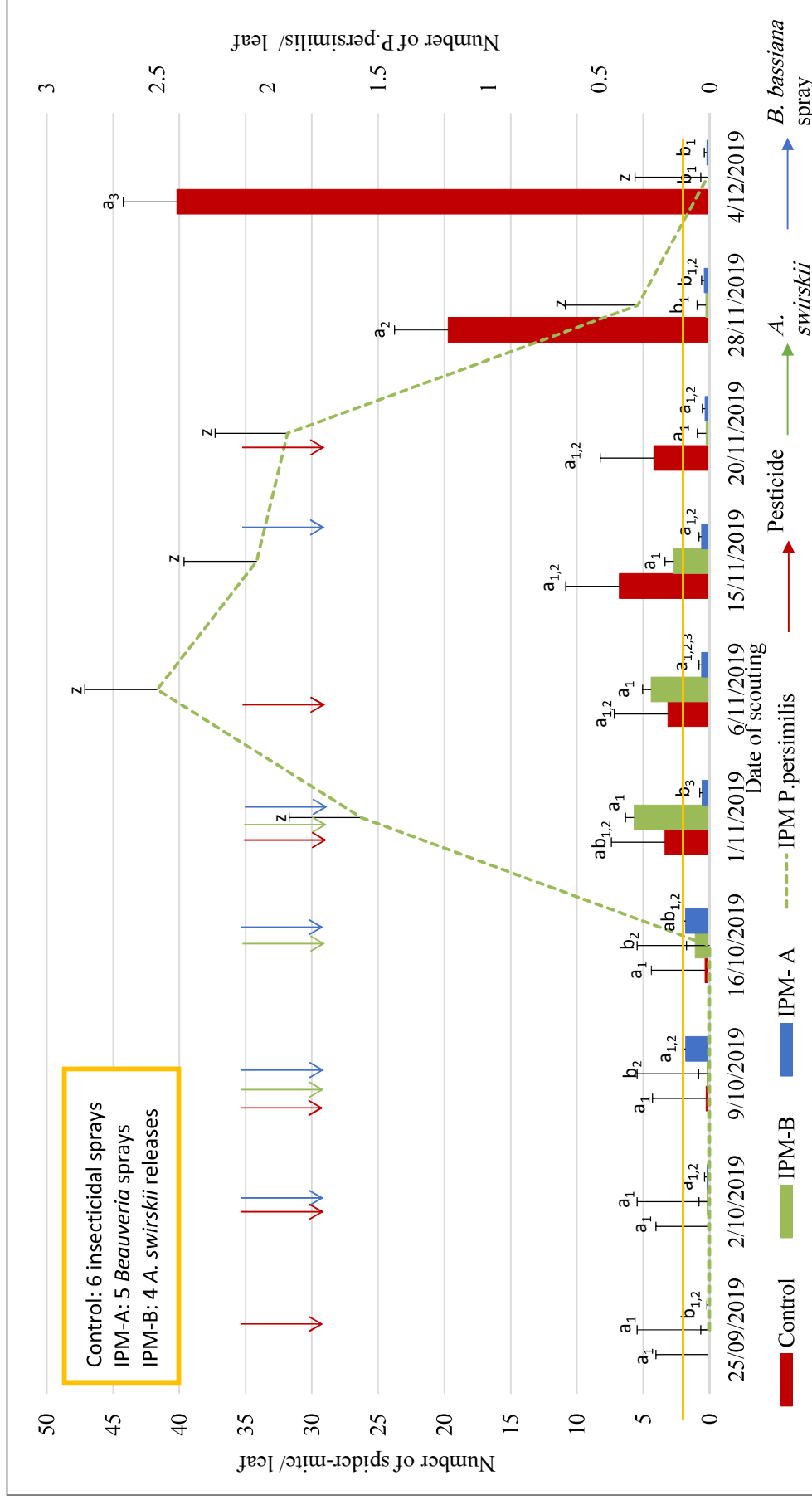


Figure 7. Average spider-mite and *P. persimilis* population recorded on cucumber leaves throughout the growing period in the control, IPM greenhouses A and B at Rmeileh. A total of 6 insecticidal/acaricidal sprays, 5 *Beauveria* sprays and 3 releases of *P. persimilis* were performed in control greenhouse, IPM greenhouses A and B, respectively. SM/L: Spider mite per Leaf; ETL: Economic threshold level. Different letters indicate statistically different values within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).

#### 4.1.5. Aphids management

In the control greenhouse, the aphids were completely absent on cucumber throughout the growing season (Fig. 8). The applied insecticides prevented any emergence and spread of aphids.

Likewise, the aphid population was almost negligible in IPM-A greenhouse. Five applications of *B. pseudobassiana* provided sufficient control of aphids all through the experiment (Appendix I, Table 1). *M. persicae* populations were almost negligible all through the season. The obtained results of the local *Beauveria* strain are comparable to other commercial ones. A 100% mortality rate among *M. persicae* populations was obtained upon the exposure to  $6.75 \times 10^5$  conidia/ml of *B. bassiana* strain 202 (Bb-202) (Bugti et al., 2018).

In the IPM-B greenhouse, the aphid population was first observed during the 5<sup>th</sup> week of the growing season early in November (Fig. 8). It must be mentined that our inability to access the experimental site during the last week of October might have delayed any necessary earlier intervention. To control aphids, the spray of *B. pseudobassiana* suspension was applied on the 5<sup>th</sup> week. Since the infestation of aphids was mainly restricted to the entrance area, a hotspot treatment of *B. pseudobassiana* was done from the middle section of the greenhouse towards the infested part. The same procedure was performed consecutively, restrciting the fast multiplication of the aphids. However, the absence of one *Beauveria* spray during week 8 along with the presence of favorable climatic conditions, allowed for the rapid multiplicaion in aphid population that continued until the end of the experiment. The delay in *Beauveria* spray until high population of aphids were established may have lead to the inefficacy of the fungus in the managemnt of high infestations.

*B. bassiana* have been commercially developed and is widely used in the control of aphid species on greenhouse vegetable crops (Prince and Chandler; 2020). However, a number of challenges still exist. The outbreak in aphids infestation in IPM-B might be attributed to the complex nature of the insect. The success of pest control by fungal pathogens is dependent on the ability to deliver lethal doses of spores to the target. However, this can be particularly difficult in the case of aphids. These pests are largely sedentary and feed by sucking sap from phloem tissues. Hence, they do not acquire fungal inoculum from treated surfaces as fast as other active, chewing pests (Wright et al. 2016). Additionally, immature aphids undergo four short intermoult periods before reaching adulthood, which removes external fungal spores that haven't penetrated the haemocoel yet, thus conferring a significant level of protection against infection (Mohammed et al. 2018). In fact, the exuviae of *Beauveria* treated *M. persicae* nymphs was observed on greenhouse cucumber seven days post infection, suggesting that the nymphs might have shed their cuticle before the fungal penetration (Shipp et al. 2003). In light of this, the sufficient control of aphids appears to be strictly reliant on the early control intervention and consistent weekly applications. In our experiment, we missed on at least one of the two factors, which have possibly led to the subsequent outbreak.

Another factor might be linked to unfavorable field conditions. Given that the efficiency of *B. bassiana* is dependent on environmental conditions, it might have been affected by unsuitable temperatures. Many isolates of *B. bassiana* performed well within a range of 20°C to 30°C, with most isolates being between 25°C to 28°C (Shipp et al. 2003). In the greenhouse, average temperatures from Mid-November onwards fluctuated between 21°C and 15.4°C, which might have reduced the efficiency of the last 3 *Beauveria* treatments.

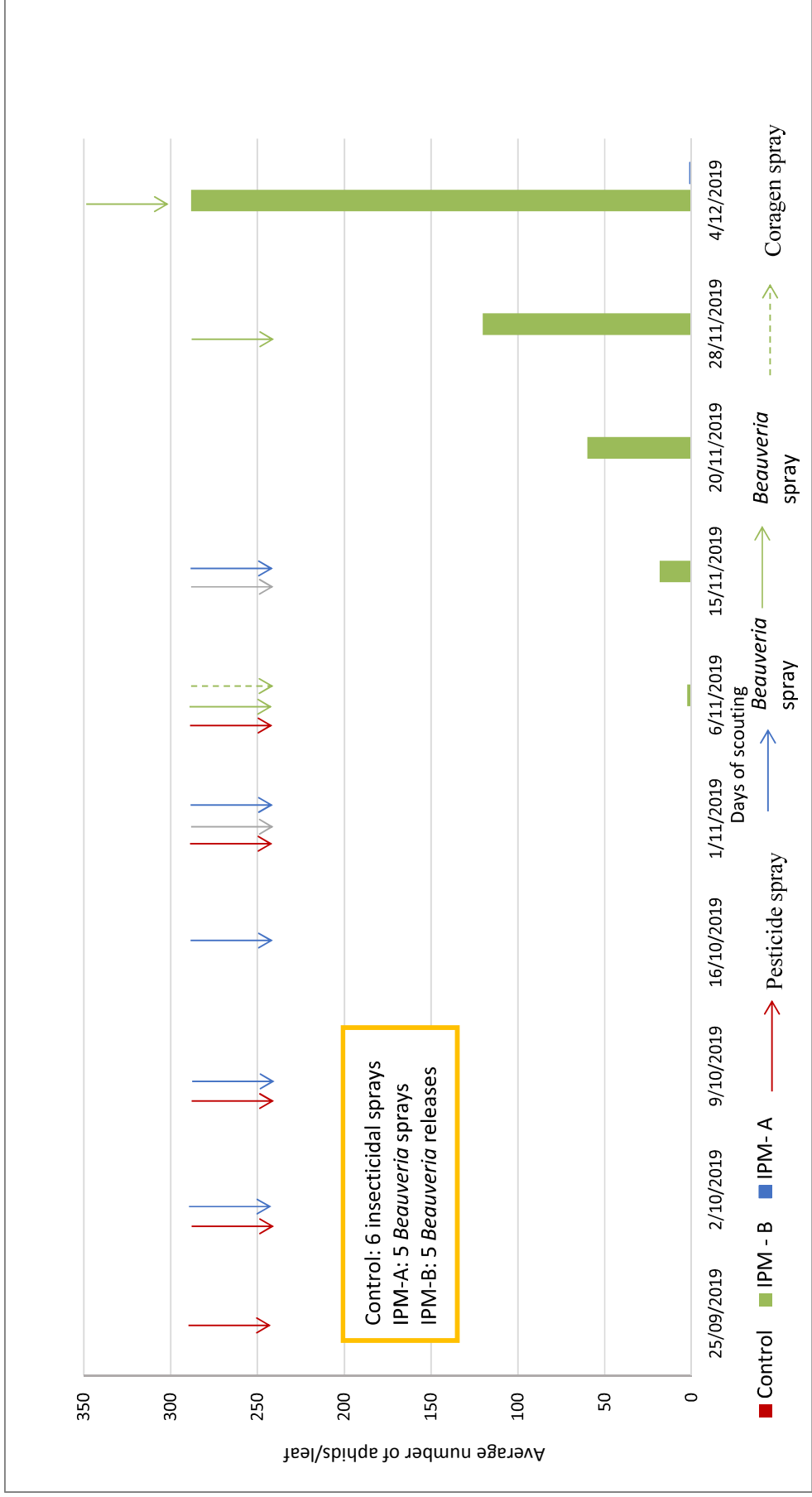


Figure 8. Average aphids population recorded on cucumber leaves throughout the growing period in the control, IPM greenhouses A and B at Rmeileh. A total of 6 insecticidal/acaricidal sprays, 5 *Beauveria* sprays and 5 hotspot *Beauveria* sprays were performed in control greenhouse, IPM greenhouses A and B, respectively. Different letters indicate statistically different values within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).



## **4.2. Large scale field trials in commercial greenhouses to evaluate the efficacy of the local fungus *Beauveria pseudobassiana* and *Bacillus thuringiensis* against tomato pests within an integrated pest management system**

### ***4.2.1. Ambient conditions***

The retrieved data on temperature and RH variations in the tomato trial are presented in Appendix II, figures 15 and 16. Throughout the growing period of the trial which was conducted during the winter/spring season (January to April 2020), the mean daily temperatures varied from 25.47°C on week 2, 14.79°C on week 6 and 21.3°C on the final week (minimum and maximum mean temperatures being 7.5°C and 38.86°C, respectively). The average relative humidity fluctuated between 37.56 and 95.38%.

### ***4.2.2. Population dynamics of pests in control and IPM greenhouses***

For the following field trial, results of the population dynamics of arthropod pests; *T. absoluta* eggs and larvae as well as leaf miner (*Liriomyza spp.*) in the control and the two IPM greenhouses (IPM-A (*B. pseudobassiana*) and IPM-B (*B. thuringiensis*), are presented in Figures 9-11.

### ***4.2.3. *Tuta absoluta* egg and larval population management***

In the control greenhouse, the farmer performed 10 pesticide sprays during the season to control arthropod pests on tomato, each of which comprised 3 active ingredients (Appendix I, Table 2). However, later after the end of the experiment, the farmer reported a mistake in the applied number of sprays, which was 20 instead of 10. Due to the lack of information on the exact date of spraying, the remaining 10 sprays were not added to the graphs. During the first 6 weeks of the experiment, average *T. absoluta* eggs population was relatively low (<0.5 egg/leaf) with slight fluctuations.

With the increase in temperature in the beginning of March, on the 7<sup>th</sup> week, a rapid increase in population by 4-folds was recorded (Fig. 9). Although this rise was accompanied by at least 1 or 2 insecticidal sprays per week until the end of the season, an exponential growth persisted reaching a peak of 3.83 eggs/leaf on the last week (Fig. 9). There was a statistically significant difference between the number of eggs on week 7 and the first 5 weeks ( $F=34.012$ ,  $df= 10$ ,  $P_{1,5} < 0.05$ ), and between the last two weeks compared to the rest the experiment ( $F=34.012$ ,  $df= 10$ ,  $P_{1,9} < 0.05$ ). In parallel, the larval population was maintained below 1 larvae/leaf until the last week of March; week 9. Afterthat, the population increased within two weeks from 0.76 to 3.6 larvae/leaf at the end of the season (Fig. 10). Throughout the season, the farmer used Emamectin benzoate (5%), Abamectin, Acetamiprid (20%) and chlorfenapyr. Yet, up to a certain time, none of them was capable of suppressing *T. absoluta* egg and larval population.

In the IPM-A greenhouse, a total number of 5 sprays (4 *B. pseudobassiana* + 1 *B. thuringiensis*) provided adequate control of *T. absoluta* eggs (Appendix I, Table 1). *Tuta absoluta* population was low during the first five weeks, so it was enough to perform selective removal of infested leaves with larval mines as a key step in cultural control within IPM to limit the development and spread of the pest. As few adult moths started to appear during the beginning of March, the first spray of *B. pseudobassiana* was done, followed by two consecutive weekly sprays. It must be noted that *B. thuringiensis* was sprayed once after that to eliminate any possible chance of phytotoxicity. By then, average egg population remained below 1 egg/leaf until the end march. An increase in population to 1.35 egg/leaf was observed on week 10, but dropped within 1 week by 70.3% in response to the application of *B. pseudobassiana* to 0.4 egg/leaf at the end of the season (Fig. 9). The larval population remained low till mid-March (Fig. 10).

Thereafter, the average population increased during the last 3 weeks of the season reaching an average of 1.9 larvae/leaf, but at lower densities and development rate compared to the control greenhouse.

In the IPM-B greenhouse, *B. thuringiensis* was used to control *T. absoluta* populations. A similar growth pattern of *T. absoluta* eggs to that of IPM-A was observed. Removal of infested leaves was done on the first few weeks as low populations persisted (Fig. 9). The first spray of *B. thuringiensis* on the first of March was performed, and repeated weekly until the end of the month. Consequently, the average population was maintained below 1 egg/leaf until week 9, and then increased on the last week of March to 1.45 egg/leaf. However, with the last spray of *B. thuringiensis*, the number of eggs fall by more than 50% to 0.6 egg/leaf on the last week (Fig. 9). Similarly, the application of *B. thuringiensis* maintained the number of larvae at low levels till the last week of March. Thereafter, the population increased gradually to reach 2.6 larvae/leaf by the end of the season (Fig. 10).

A significant difference was observed between treatments ( $F_{eggs} = 61.633$ ,  $df = 2$ ,  $P < 0.01$ ;  $F_{larvae} = 14.342$ ,  $df = 2$ ,  $P < 0.01$ ), weeks ( $F_{eggs} = 59.029$ ,  $df = 10$ ,  $P < 0.01$ ;  $F_{larvae} = 97.041$ ,  $df = 10$ ,  $P < 0.01$ ) as well as treatment\*week ( $F_{eggs} = 15.034$ ,  $df = 18$ ,  $P < 0.01$ ;  $F_{larvae} = 3.262$ ,  $df = 20$ ,  $P < 0.01$ ). The use of biocontrol agents' *B. pseudobassiana* and *B. thuringiensis* provided superior control of *T. absoluta* compared to chemical insecticides. During the last five weeks of the season, the average number of egg population was significantly higher in the control than that in IPM-A and IPM-B greenhouses, whereas the difference between that latter two greenhouses was insignificant. The maximum number of *T. absoluta* eggs was recorded on the last week in the control greenhouse; 3.8 eggs/leaf. This is compared to 0.4 and 0.6 eggs/leaf in IPM-

A and IPM-B, respectively. Compared to the control, the difference corresponds to 89.5 and 80% reduction in IPM-A and IPM-B, respectively. Similarly, a higher number of larvae was recorded in the control compared to IPM-A and IPM-B. A statistical difference was observed between the control and IPM-A greenhouse corresponding to 47.2% reduction. Likewise, the population was lower in IPM-B with respect to the control by 27%, yet not statistically significant.

The population of *T. absoluta* increased consistently during the last 5 weeks of the trial starting March 10 in all greenhouses. The average temperature during the first 6 weeks of the trial (January 28-March 3) fluctuated between 25.47 and 16.1° C. Maximum and minimum temperatures were 38.5 and 9.01° C, respectively. Meanwhile, average temperatures during the last 5 weeks varied between 22.75 and 13.71° C; corresponding to lower mean temperatures by 3° C compared to the earlier period. Maximum and minimum temperature were 38.86 and 7.5° C, respectively. Although thermal requirements for *T. absoluta* development varied with different locations and strains, a common range for optimum temperature is 20-30°C. Lower and upper thermal thresholds have been established at 8°C and 37.7°C, respectively (Krechemer et al. 2015). In the current study and according to the thermal requirements of *T. absoluta*, the variations in temperature between the beginning and at the end of the trial don't seem significant as they appear. However, further analysis of this data based on the relationship between accumulated thermal units and population fluctuation may reveal some effects (Salama et al. 2019).

#### 4.2.4. Leaf miner (*Liriomyza spp.*) management

In the control greenhouse, the development curve of leafminer doesn't show any clear pattern. The average population fluctuated throughout the experiment below 0.5 miner/leaf and peaked part way through the season reaching 0.69 miner/leaf . The applied insecticides maintained the population at 0.125 miner/leaf at the end of the season (Fig. 11).

By comparison, average densities of leafminer remain relatively low in IPM-A and IPM-B till the end of the experiment; 0.025 miner/leaf. A significant difference was observed between treatments ( $F = 42.643$ ,  $df = 2$ ,  $P < 0.01$ ), weeks ( $F = 53.01$ ,  $df = 10$ ,  $P < 0.01$ ) as well as treatment\*week ( $F = 25.021$ ,  $df = 18$ ,  $P < 0.01$ ). On the final week, the leafminer population was lower by 80% in both IPM greenhouses as compared to the control, although insignificant. *B. thuringiensis* provided the best control of leafminer throughout the season.

There have been few attempts to evaluate entomopathogens and microbial agents against dipteran leafminers. Under lab conditions, treatments with *B. bassiana* clade C + Tween 80 resulted in 73-93% mortality in the pupal stage of *Liriomyza huidobrensis* (Noujeim et al., 2015). The susceptibility of *L. huidobrensis* adults to *Beauveria* was also reported (Migiro et al. 2010). Under field conditions, *B. thuringiensis* was shown to infect the larval stages *Liriomyza sativae* on tomato and *Liriomyza trifolii* on beans (Çıkman and Çömlekçioğlu, 2006). Herein, we demonstrate the insecticidal activity of *B. pseudobassiana* and *B. thuringiensis* against leafminer (*Liriomyza spp.*) on tomato under commercial greenhouse conditions.

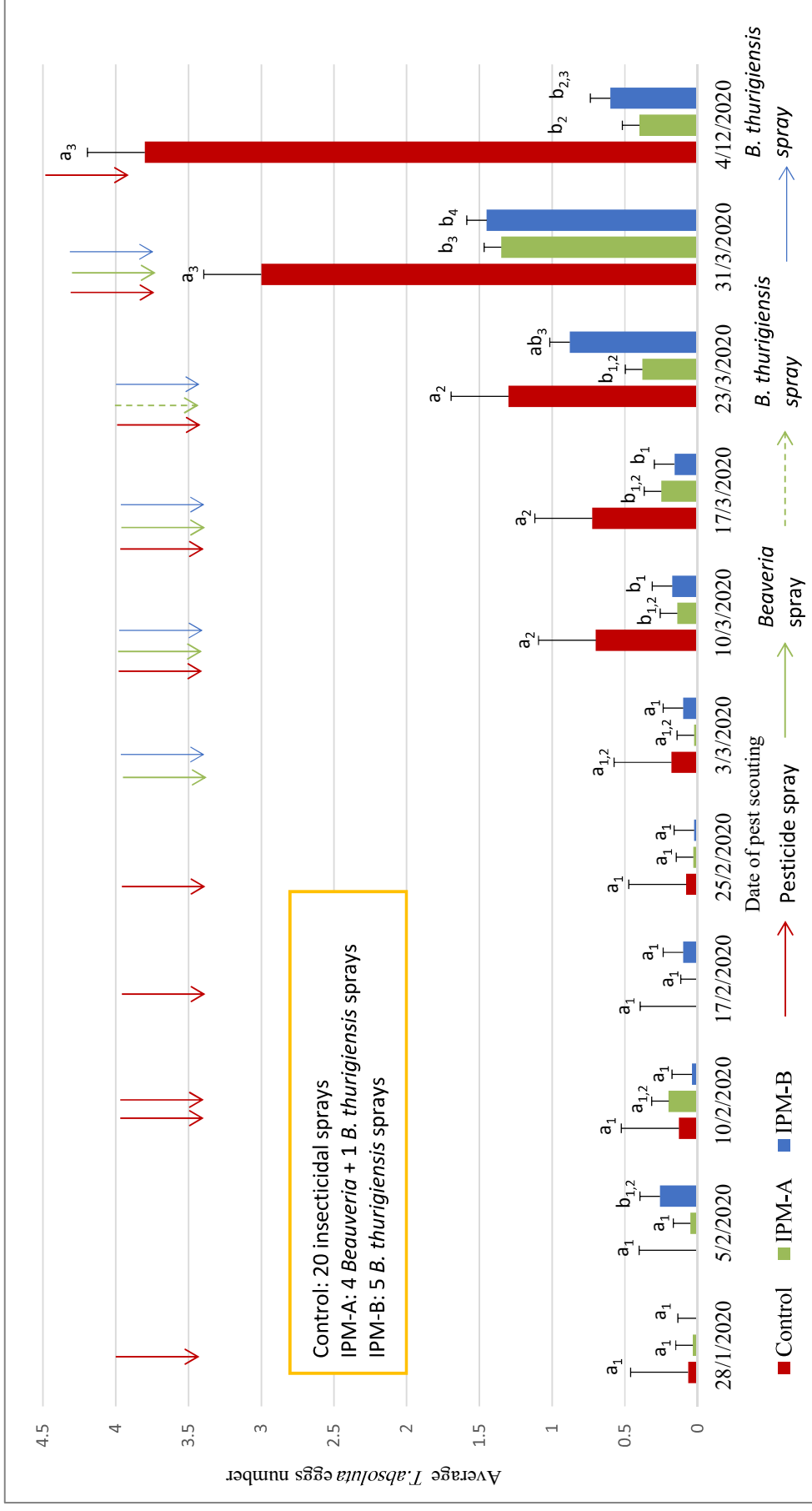


Figure 9 Average *T. absoluta* eggs population recorded on tomato leaves throughout the growing period in the control, IPM greenhouses A and B at Rmeileh. Control: A total of 20 insecticidal/acaricidal sprays, IPM A: 5 *Beauveria* + 1 *B. thuringiensis* sprays, and IPM B: 5 *B. thuringiensis* sprays were performed. Different letters indicate statistically different values between each treatment within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).

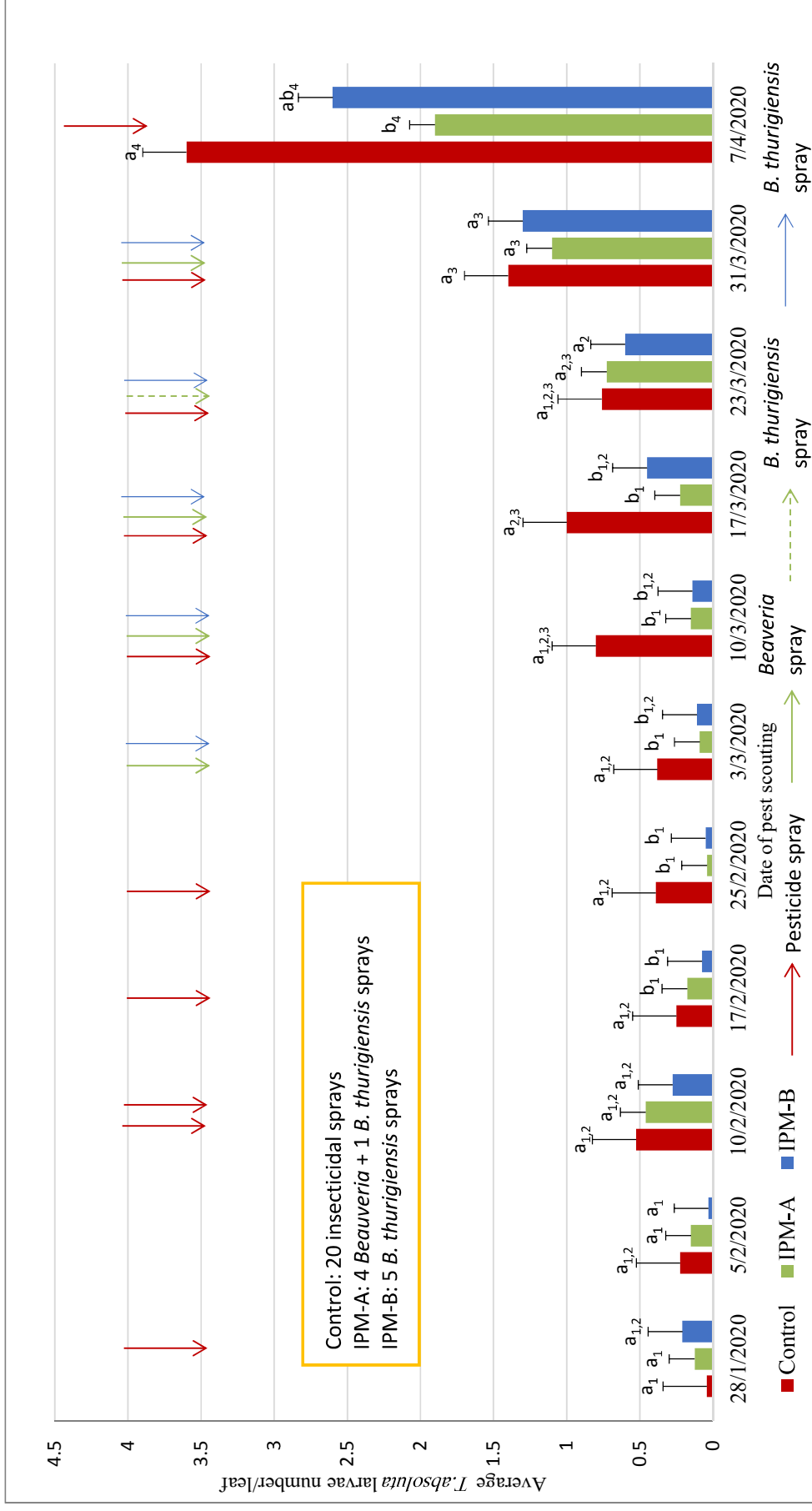


Figure 10 Average *T. absoluta* larval population recorded on tomato leaves throughout the growing period in the control, IPM greenhouses A and B at Rmeileh. Control: A total of 20 insecticidal/acaricidal sprays, IPM A: 5 *Beauveria* + 1 *B. thuringiensis* sprays, and IPM B: 5 *B. thuringiensis* sprays were performed. Different letters indicate statistically different values between each treatment within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).

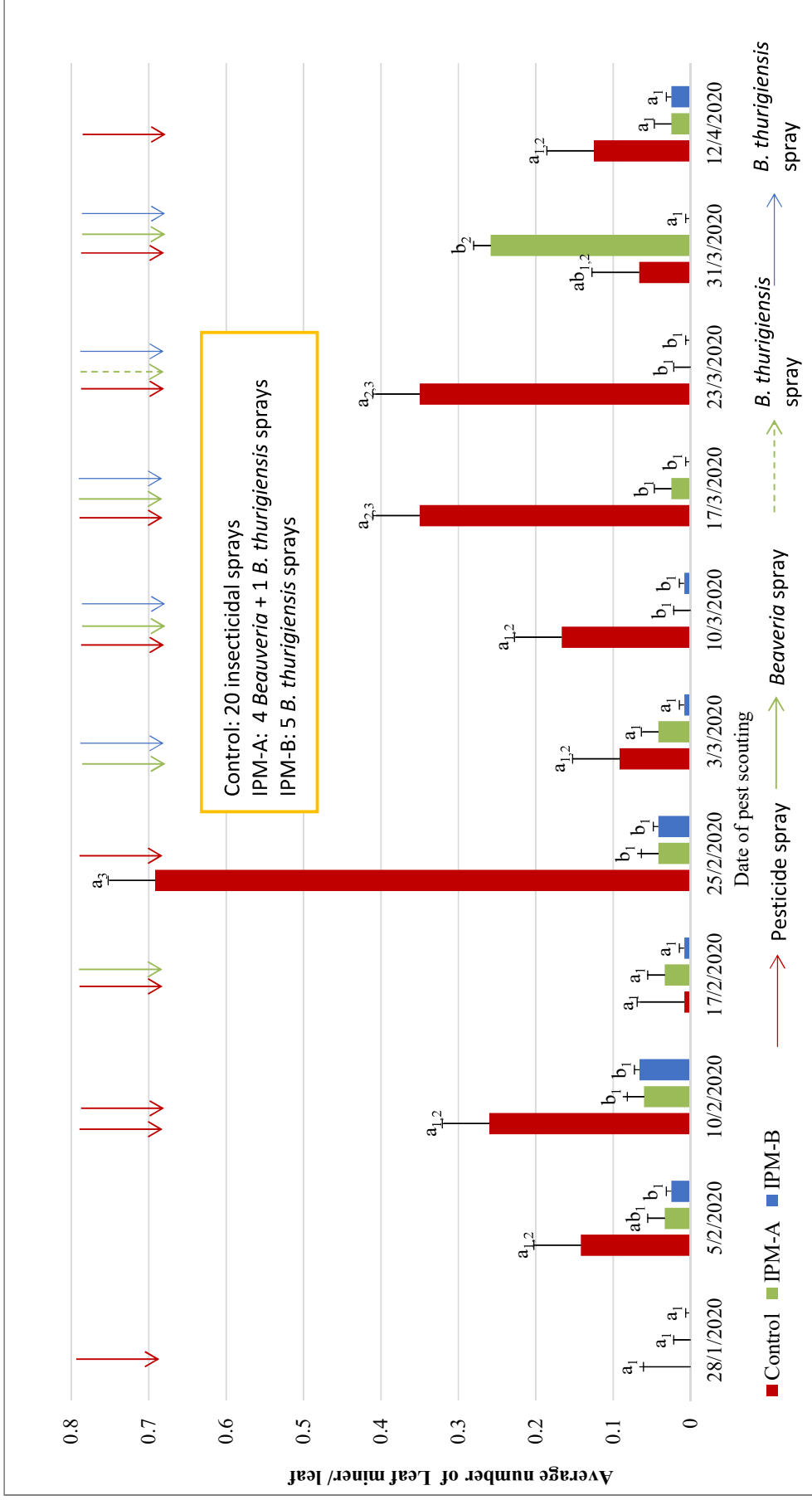


Figure 11 Average leaf miner population recorded on tomato leaves throughout the growing period in the control, IPM greenhouses A and B at Rmeileh. Control: A total of 20 insecticidal/acaricidal sprays, IPM A: 5 *Beauveria* + 1 *B. thuringiensis* sprays, and IPM B: 5 *B. thuringiensis* sprays were performed. Different letters indicate statistically different values between each treatment within each week based on Tukey's HSD ( $P < 0.05$ ). Different numbers indicate statistically different values within each treatment based on Tukey's HSD ( $P < 0.05$ ).



#### **4.2.5. Chemical control**

The obtained results showed an inefficiency of chemical control against *T. absoluta*. The development of insecticide resistance among *T. absoluta* populations has been monitored over the years, highlighting fast and global resistance levels to major classes of insecticides including organophosphates, pyrethroids, pyrethrins, spinosyns, carbamates, diamides, and oxadiazines (Biondi et al. 2018; Guedes et al. 2019; Bala et al. 2019). However, an exception to the intensified spread of resistance cases of *T. absoluta* is the class of avermectins (Inak et al. 2021). To date, despite a single case of resistance to abamectin in Brazil, no reports of resistance to avermectins have been documented (Siqueira et al. 2000; Inak et al. 2021). Additionally, no cases of control failure of the tomato leaf miner have been associated with the use of emamectin benzoate, a second generation avermectin (Roditakis et al. 2018). In fact, recent tomato field trials in Tunisia and Egypt revealed high efficacy of the insecticide describing it as the most potent on *T. absoluta*; 90% mortality rates and 98.74% reduction in infestation levels, respectively (Simmons et al. 2018; Kandil et al. 2020). Similarly, no resistance of *T. absoluta* was detected against chlorfenpyr, which provided sufficient control of the pest (Guedes et al. 2019; Kandil et al. 2020). On the contrary, the conducted experiment showed a limited efficacy of the mentioned insecticides against *T. absoluta* in the farmers' greenhouse although frequent applications were performed (20 sprays per season). This might be attributed to different factors, one of which suggest developed resistance of the local strain of *T. absoluta* against these chemicals.

#### 4.2.6. IPM greenhouses

The findings of the presented commercial greenhouse IPM-based trails confirm the effectiveness of both *B. pseudobassiana* and *B. thuringiensis var. kurstaki* formulations against *T. absoluta* on tomato. This study demonstrated that the sole application of either of the two biocontrol agents is capable of reducing the pest's impact to low levels without the input of chemical insecticides. The use of the local isolate *B. pseudobassiana* outperformed chemical insecticides by significantly reducing *T. absoluta* populations. Similarly, *Bt*-treated tomato showed lower densities of eggs and larvae compared to the control, although it was insignificant among larval instars. Despite the national lockdown due to COVID-19 pandemic that took place from March 15 till the end of the field trial (April 7), affecting our weekly field scouting/sprays, both IPM greenhouses provided superior pest control compared to the control greenhouse.

Recently, a study evaluated the efficacy of *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus thuringiensis* against *T. absoluta* either applied solely, mixed together or combined with Tutan 36% SC (Chlorphenapyr) in field trials under natural pest infestation incidences. Insecticide, Tutan36% SC was used as standard control. Sprays were done twice per week in the first month and once per week in the following months. The spraying frequency is relatively high compared to our trial. All bio-control agents outperformed the control; more than 80% reduction in leaf and fruit infestation, with *Beauveria* being the most effective. The combined treatments appeared more effective against *T. absoluta* than the single treatments. Our results also confirm that the control potential of *B. thuringiensis var. kurstaki* against *T. absoluta* was intermediately significant compared to *Beauveria*, yet higher than the control. The variation could be due to environmental factors, degradation of toxins and/or toxin inactivation by enzymes

produced by the pest. Additional combined effects were not assessed as it was beyond the scope of our study. Other reports had, however, shown significantly higher fruit protection when *B. thuringiensis* and *B. bassiana* were combined with Azadirachtin, this suggests the combination of multiple biopesticides might improve the efficacy (Jallow et al. 2019).

#### **4.2.7. *Beauveria*: Lab, greenhouse and field experiments**

Pathogenicity of different strains of *B. bassiana* on both egg and larvae of *T. absoluta* has been demonstrated by several studies under lab, greenhouse and semi-field conditions (Sabbour and Sahab, 2005; Tadele et al., 2017; Abdel-Baky et al., 2021; Buragohain et al., 2021). The findings of the current field trial showed that the use of local *B. pseudobassiana* strain had the highest control against *T. absoluta* compared to chemical insecticides and *B. thuringiensis*-based formulation. In particular, a significant reduction among egg (89.5 %) and larval populations (47.2%) was observed with respect to the control.

The use of *B. bassiana* has had its own constraints (Dannon et al., 2020). In vitro, the death of an insect took 3 to 5 days' post fungal application. This time interval may be considerably greater in the field due to suboptimal conditions that lead to an extended disease initiation and progression (Strasser, 2001). An increase in temperature by 3 to 5 degrees delays the time of death by 1 day which is critical in the management of the cryptic stages of *T. absoluta* such as larvae and pupae. (Strasser, 2001). The activity of *B. bassiana* appears to be hampered by elevated temperatures and low RH levels. Recently, a study aiming to identify thermotolerant *B. bassiana* isolates showed that 11 strains collected from different areas of Syria were able to germinate and grow at

temperatures of 20, 25 and 30 but not at 35 °C (Alali et al., 2019). Similarly, temperatures above 36°C did not support the growth of *B. bassiana* (Buragohain et al., 2021). A 100% RH was optimal for mycelial growth and germination, although some strains tolerated lower levels (56.8%) (Dannon et al., 2020). The maximum and minimum temperatures recorded throughout the experimental period were 38.7 and 7.519°C, respectively. In fact, maximum daily temperatures above 36°C were frequently observed from late February until the end of the season. Minimum RH levels reached 33% by Mid-February and mean RH ranged between 44 and 73% during the whole experiment. Hence, the fungal efficacy against larval stages might have been affected.

#### **4.2.8. *B. thuringiensis*: Lab, greenhouse and field experiments**

The use of *B. thuringiensis* became a key component in integrated pest management (IPM) (Sarr et al., 2020). In addition to Lepidoptera, the pathogenicity of *B. thuringiensis* was reported on an array of economic pests, including whiteflies, mites and thrips and *Lirimyza spp.* *Bt*-based formulations have been widely used for the control of *T. absoluta*, being the most effective bioinsecticide in reducing damage to tomato leaves and fruits in field conditions (Tarusikirwa et al., 2020). In this study, the use of *B. thuringiensis* caused a significant reduction in egg population (80%) but not among larvae (27%) of *T. absoluta*. Surprisingly, a greater impact was observed on *T. absoluta* eggs compared to the larvae. Studies on the effect of *B. thuringiensis* on eggs are limited since it's mainly used as a larvicidal bioinsecticide. Our results are in agreement with those of Alwan et al. (2012) who found a significant decrease in the percentage of *T. absoluta* egg hatching treated with *B. thuringiensis* filtrate (33.36%) compared to the control (86.74%). Indeed, the use of bacterial isolates interfered with egg formation, hence reducing the

number of laid eggs. This phenomenon showed that some larvae may be slightly infected, and require more time to attack stomach cells which would delay lethality or infection symptoms to later stages of pupae or adults (Youssef and Hassan, 2013). To some extent, this might explain the limited impact of *Bt* on larvae in our experiment which could have been visible on older stages had the trial been extended.

Various studies confirmed high control potential of commercial Bt-formulations against *T. absoluta* larvae in laboratory, greenhouse, and open field conditions (Mansour et al. 2018; Sarr et al. 2020). The success of *B. thuringiensis var. kurstaki* strains in field conditions varied due to application mode, environmental factors, toxin degradation, and target characteristics such as exposure time, dose, and larval stage (although all stages are affected) (Abd El-Ghany et al. 2021). Theoretically, since the larvae should be outside the mine to collect a lethal dose of the product, the free living phase of the first instar larvae is assumed to be the most vulnerable. At a constant temperature, it has been found that the first instar spends 82 minutes only on the leaf surface corresponding to 0.005% of the larval development. Hence, frequent application of *B. thuringiensis* (less than weekly intervals) was suggested (Cuthbertson et al., 2013). Mature larvae bore into the tomato leaves, fruits and flowers, spending most of their lifespan inside the crop. This concealed feeding behavior allows the pest to escape from applied insecticide or/and bio insecticide (Agbessenou et al., 2020). El-Ghany et al. (2016) found a weak larval mortality effect when testing different strains of *B. thuringiensis kurstaki* which was linked to higher sensitivity of Bt to environmental conditions and/or the pest nature. Despite of the common limitations, the use of *B. thuringiensis* in combination with other products or predators, remains an integral part within and IPM program against *T. absoluta*.

Many authors have reviewed the natural enemy complex of *T. absoluta*. Among these species, two predatory mirid bugs, *Macrolophus pygmaeus* and *Nesidiocoris tenuis*, have been found in the near east region of Lebanon (FAO, 2016). Indigenous natural enemies need time to adapt, develop and establish in order to provide an effective control of exotic pests (Buragohain et al., 2021). Hence, augmentative biological control is implemented within an IPM approach in the aim of conserving both released and naturally occurring species of natural enemies (Gonzalez et al., 2016). Since chemical or biological compounds used against *T. absoluta* could directly or indirectly affect its natural enemies, it is necessary to select suitable products in the pest management programs. In this context, the use of *B. bassiana* and *B. thuringiensis* appears promising. Interestingly, after studying the effects of *B. bassiana* on the survival of *N. tenuis*, Assadi et al. (2021) recommended the combined use of the both agents while keeping a time interval between the two introductions. Similarly, the use of *B. thuringiensis* was safe, effective and compatible with both *N. tenuis* and *M. pygmaeus* (Mollá et al., 2011; De Backer et al., 2014).

#### **4.3. Compatibility of the local *B. pseudobassiana* and *A. swirskii* strains for the management of whitefly in a small scale greenhouse using insect-proof cages**

This experiment aimed at studying the compatibility between two natural enemies for the management of the whitefly *Bemisia tabaci*, by evaluating the efficacy of the sole and combined applications of *A. swirskii* and *B. pseudobassiana* for the management of whitefly nymphs under insect-proof greenhouse conditions.

The experiment was conducted in a glasshouse at an average temperature of 25-28°C and 60-70% RH. On cucumber, whitefly nymphs emerged on the next week following the initial release of adults. Therefore, a single release of *A. swirskii* was done on the 2<sup>nd</sup>

week of the experiment, along with two sprays of *Beauveria* and oil on the 2<sup>nd</sup> and 3<sup>rd</sup> week in the respective treatments. A second whitefly release was performed on week 4. During the 2<sup>nd</sup> and 3<sup>rd</sup> week, a gradual increase in whitefly nymphs was observed in all treatments. Then, on week 4 the population increased significantly by 4 folds in the control and oil treatments. Following the next two weeks, the nymphal density was almost constant in the control and decreased slightly in the oil treatment. However, it peaked on the last week reaching 11.62 and 13.5 nymphs/leaf in the oil and control treatments, respectively.

While the nymphal population grew on week 4 in the control and oil treated plants, it dropped significantly in *A. swirskii*, *B. pseudobassiana* and *A. swirskii*+ *B. pseudobassiana* treatments. In the *B. pseudobassiana* treatment, the number of nymphs dropped 1 week post the second spray from 2.48 to 0.77 nymphs/leaf. This average was maintained at a similar level with a very slight increase during each week to reach 1.85 nymphs/leaf at the end of the experiment. Meanwhile, the nymphal population decreased progressively throughout the experiment in *A. swirskii* and *A. swirskii*+ *B. pseudobassiana* treated plants, but at higher rates in the latter treatment. One week post the release of *A. swirskii*, the mite population peaked reaching an average of 5.67 and 5.88 mite/leaf in *A. swirskii* and *A. swirskii*+ *B. pseudobassiana*, respectively. Afterwards, the number of nymphs started to decrease, and continued until the last week to reach 0.29 nymphs/leaf in *A. swirskii* treatment and 0.11 nymphs/leaf in *A. swirskii*+ *B. pseudobassiana* treatment.

A statistically significant effect was observed in the average number of whitefly nymphs between treatments ( $F = 792.966$ ,  $df = 4$ ,  $P < 0.01$ ), weeks ( $F = 812.956$ ,  $df = 6$ ,  $P < 0.01$ ) as well as treatment\*week ( $F = 236.826$ ,  $df = 24$ ,  $P < 0.01$ ). From the 4<sup>th</sup> week

onwards, the number of whitefly nymphs was significantly higher in both the control and oil treatments compared to *A. swirskii*, *A. swirskii*+ *B. pseudobassiana* and *B. pseudobassiana* treatments. By comparison, the maximum number of whitefly nymphs was recorded on the last week in the control greenhouse; 13.5 nymphs/leaf. This is compared to an average of 11.62, 1.85, 0.29 and 0.11 nymphs/leaf in the oil, *B. pseudobassiana*, *A. swirskii*, and *A. swirskii*+ *B. pseudobassiana*, respectively. Relative to the control, the difference in the whitefly nymphs represents a reduction of 14 % in the oil treatment, 86.3 % in *B. pseudobassiana* treatment, 97.85 % in *A. swirskii* treatment, and 99.2 % in *A. swirskii*+ *B. pseudobassiana* treatment.

There was no statistical difference between the average number of *A. swirskii* in *A. swirskii* and *A. swirskii*+ *B. pseudobassiana* treatments. The population of the mite grew similarly all throughout the experiment in both treatments. This shows that *A. swirskii* wasn't affected by the two sprays of *B. pseudobassiana* which confirms that the application of the two biocontrol agents simultaneously is compatible and provides high efficacy against whitefly nymphs. In fact, not only did the combined treatment provide optimum suppression of whiteflies, but also recorded a higher average in *A. swirskii* population compared to the *A. swirskii* treatment alone.

Our results showed that the two local strains of *A. swirskii* and *B. pseudobassiana* are efficient in the control of *B. tabaci* nymphs. A single application of *A. swirskii* at a rate of 50 mite/cage reduced the number of immature *B. tabaci* by 97.85 % compared to an untreated control five weeks after the release. Similarly, two sprays of *B. pseudobassiana* applied at 1 week interval suppressed the nymphal population by 86.3 % relative to the control. The concomitant use of both agents had the highest reduction level against *B. tabaci* over the control reaching 99.2 %. Most studies on the



interactions between entomopathogenic fungi and predators, in particular *A. swirskii* and *B. bassiana*, have been laboratory-based characterizations of negative interactions, such as infection of the mite by fungi, or predation of fungal-infected hosts. For instance, under lab conditions adults' *A. swirskii* were found to be susceptible to commercial *B. bassiana* strain GHA, yet there was no negative effects on juvenile survival (Midthassel et al., 2016). At similar conditions, Seiedy et al. (2015) found that the susceptibility level of *A. swirskii* to *B. bassiana* is strain dependent. While the fungal isolate F caused high mortality rates among the mite populations, isolate DEBI008 was compatible with the mite and recommended for the use in the control of *T. vaporariorum*. Few field scale investigations have been conducted to assess interaction possibilities between these natural enemies. Under glasshouse conditions, the use of *B. bassiana* and *A. cucumeris* was effective against *F. occidentalis* (Jacobson et al., 2010). Based on our results, the combined use of *A. swirskii* and *B. pseudobassiana* can be an effective IPM package against *B. tabaci*. Until now, previous work recommended the complementary use of the two BCAs: *A. swirskii* released in a preventative manner and later reinforced by *B. pseudobassiana* at high pest densities. Herein, we confirm the possibility of simultaneous applications of *A. swirskii* and *B. pseudobassiana* without any detrimental effects on the predatory mite. Further work should focus on the effect of the local strain of *B. pseudobassiana* on the development of *A. swirskii*, and examine the likelihood of additive or synergistic contributions if any.

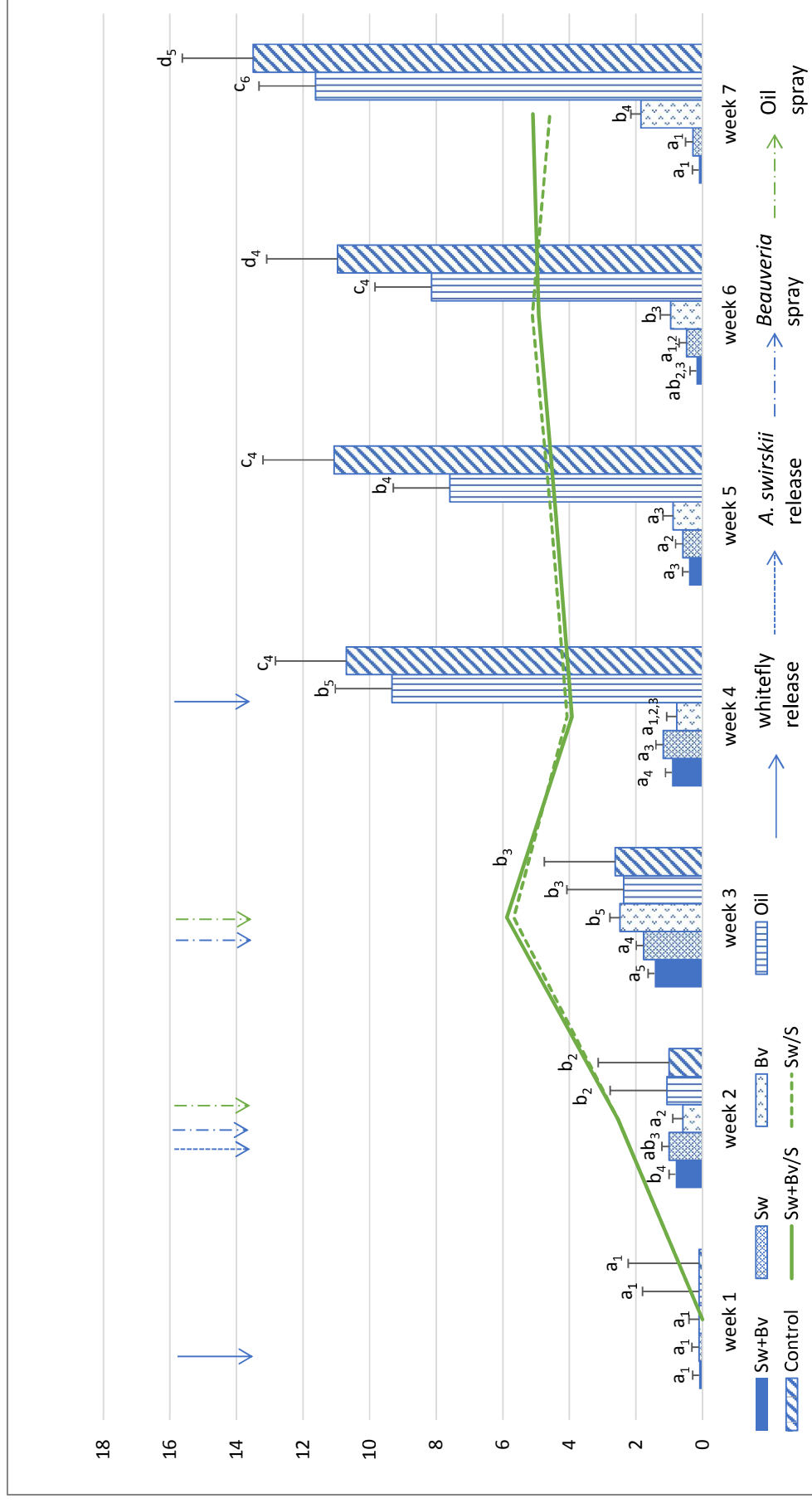


Figure 12 Average whitefly nymphs and *A. swirskii* populations recorded on cucumber leaves in different treatments: (1): *A. swirskii* (Sw/ wfn); (2): *A. swirskii* + *Beauveria* (Sw + bv/ wfn); (3): *Beauveria* (Bv// wfn); (4): Oil; (5): Control. A total of 2 whitefly releases was done in all treatments. A single release of *A. swirskii* and 2 applications of *Beauveria* and oil were performed in their respective treatments. Sw: *A. swirskii* i; Bv: *Beauveria*; Wfn: whitefly nymphs. Different letters indicate statistically different values between each treatment within each week based on Tukey's HSD (P<0.05). Different numbers indicate statistically different values within each treatment based on Tukey's HSD (P<0.05).

## CHAPTER 5

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

In Lebanon and elsewhere, pest control is still heavily reliant on chemical use in protected vegetable production. To limit the detrimental impacts on human and environment and avoid insect resistance against the receding active ingredients in the market, biological alternatives for conventional chemical control are much sought of and even urgently needed. Entomopathogenic fungi (EPFs) and predatory mites are among the most important biological agents and their use is globally increasing. Intensive studies on EPFs, mainly *Beauveria spp*, proved it equivalent to or even superior than chemical pesticides in controlling some commercially damaging pests. Likewise, multiple success stories and field trials have been associated with the use of predatory mites *A. swirskii* and *P. persimilis*. Since greenhouse crops are attacked by several pests, the integration of more than one biocontrol agent may be required. However, the efficacy and compatibility between the introduced biocontrol agents should be evaluated. Furthermore, local natural enemies may be more adapted to the local environment and may be more efficient than introduced ones. The objectives of this study were to evaluate the efficacy of locally collected natural enemies and asses the compatibility of their integrated use for the management of arthropod pests under commercial greenhouse production, a key step to improve the sustainability of agriculture production and food safety in Lebanon.

A small scale greenhouse trial in insect-proof cages included the following five treatments: (1) *A. swirskii*, (2)  $10^8$  *Beauveria* conidia mL<sup>-1</sup> + 1% corn oil + 0.01% Tween-20; (3) *A. swirskii* +  $10^8$  conidia mL<sup>-1</sup> + 1% corn oil + 0.01% Tween-20 , +

*Phytoseiulus*; (4) 1% corn oil + 0.01% Tween-20, and (5) the untreated control. The results showed that *B. tabaci* populations increased continuously in the control and oil treatments till the end of experiment. By contrast, the number of *B. tabaci* nymphs in the other three bio control treatments remained low and was significantly lower than the control by the end of the experiment. The difference in the whitefly nymphs represented a reduction of 14 % in the oil treatment, 86.3 % in *B. pseudobassiana* treatment, 97.85 % in *A. swirskii* treatment, and 99.2 % in *A. swirskii*+ *B. pseudobassiana* treatment compared to the control. In conclusion, this experiment showed that the local strain of *A. swirskii* was highly effective against whiteflies and that its simultaneous use with the local isolate of *B. pseudobassiana* is highly compatible. In fact, the concomitant use of both agents had the highest reduction level against *B. tabaci*. Hence, these two biological control agents can be used together to enhance the efficiency of IPM programs by targeting a broader spectrum of pests with an improved efficacy.

Two large scale trials in commercial size greenhouses were conducted at Rmeileh to assess the efficacy of the locally collected *B. pseudobassiana*, *P. persimilis* and *A. swirskii* against arthropod pests.

The first trial was conducted on cucumber between September and December 2019. The average temperature and average RH in the first trial which took place during the fall season were 25.14 °C (Min/max: 15/32°C) and 71.82% (Min/Max: 51/90%), respectively.

In the control greenhouse, the farmer followed his conventional protection and production practices, including the pre- and post-transplanting measures. The application of 6 sprays of broad-spectrum insecticides/acaricides have successfully suppressed thrips

and aphids pest populations on cucumber. In contrast, whitefly adult population was slightly below the ETL (5 insects/leaf), whereas the number of immatures exceeded the ETL by mid of the season causing heavy infestations. Similarly, the number of two-spotted spider mites (TSSM) increased and exceeded the ETL on the 1<sup>st</sup> of November, up until an outbreak took place on the last week reaching a level of 40.17 spider mite/leaf.

In the IPM-A greenhouse, *Beauveria* was sprayed for the control of whiteflies, thrips, aphids and spider mites. The application of 5 fungal sprays suppressed all four arthropod pests below their ETL level throughout the season. Despite the favorable environmental conditions for pest development, the local strain of *B. pseudobassiana* provided exceptional control against the four serious economical arthropod pests.

In IPM-B greenhouse, locally collected predatory mite *A. swirskii* was used for the control of whiteflies and thrips, and *P. persimilis* was used against spider mites. Hot spot applications of *B. pseudobassiana*, were performed for the control of aphids. A total number of four *A. swirskii* releases was sufficient to maintain whitefly and thrips populations below the economic threshold of 5 whiteflies/leaf (Shen et al. 2005) and 1.3 thrips/ leaf. Although maximum recorded temperatures were above 40° C until the third week of October, *A. swirskii* was able to establish and forage well. Since *A. swirskii* preyed on two different pests simultaneously within a single season, assessing the proper ratio of the mite to either of the two pests could not be performed. However, it's worth mentioning that among whiteflies, when the ratio reached 2.12 whitefly for each *A. swirskii*, the average density of adults and nymphs dropped progressively till the end of trials. In regards to thrips, our observations revealed that upon reaching a ratio of 3.33 thrips for a single *A. swirskii* mite, caused a significant decrease among thrips population within one week. Indeed, *A. swirskii* proved active and efficient while

preying on a mixed diet of thrips and whiteflies. The mite *P. persimilis* was able to successfully control TSSM populations in the presence of sufficient densities of the pest. The optimal ratio of *P. persimilis* to spider mites on greenhouse cucumbers was found to be at or below 1:4. As for aphids, the population was controlled by weekly hotspot sprays of *B. pseudobassiana*. However, the absence of one *Beauveria* spray during week 8 along with the presence of favorable climatic conditions, allowed for the rapid multiplication in aphid population that continued until the end of the experiment. Indeed, *Beauveria* sprays may be more effective at low aphids populations but less at high densities where the pest has properly established. We conclude that the efficient control of aphids appears to be strictly reliant on the early control intervention and consistent weekly applications due to the complex nature of the pest.

The second trial was conducted on tomato between January and April 2020. The, temperature and RH during the winter/spring season (January to April 2020), averaged 20.47°C (Min/Max: 13/25°C) and 74.87% (Min/Max: 37-95%), respectively.

In the control greenhouse, the farmer followed his normal protection and production practices. Although 20 pesticide sprays using 2 to 3 active ingredients per application were performed during the growing season, the farmer received the least control efficiency among *T. absoluta* eggs and larvae as well as leafminer (*Liriomyza spp.*) on tomato compared to IPM- greenhouses.

In the IPM-A greenhouse, A total number of 5 sprays (4 *B. pseudobassiana* + 1 *B. thurigiensis*) provided adequate control of *T. absoluta* eggs and larval populations. Compared to the control, a significant difference was observed corresponding to 89.5% among eggs and 47.2% among larvae. Average densities of leafminer remained low in

the greenhouse till the end of the experiment showing 80% reduction relative to the control.

In the IPM-B greenhouse, *B. thuringiensis* was used to control *T. absoluta* populations. A similar growth pattern of *T. absoluta* eggs to that of IPM-A was observed. Compared to the control, the egg population was significantly lower by 80%, whereas that of the larvae was reduced by 27%. As for leafminer, the population was lower by 80%.

In conclusion, this study showed a high potential for the use of locally collected natural enemies in the management of major arthropod pest populations under normal greenhouse conditions in Lebanon. The high efficacy of local strain of *A. swirskii* in the control of whiteflies and its compatibility for combined treatment with *B. pseudobassiana* were proven in small scale trials on cucumber. Based on these findings, commercial greenhouse trials were performed on cucumber. The application of *B. pseudobassiana* at a concentration of  $10^8$  conidia  $\text{mL}^{-1}$  + 1% corn oil + 0.01% tween-20 was highly effective in suppressing whiteflies, thrips, spider mites and aphids to below ETL and allowed 100% reduction in insecticidal/acaricidal applications. Within the same study, the use of the local strains of *A. swirskii* and *P. persimilis*, when combined with additional integrated pest management measures, proved highly effective in suppressing whitefly, thrips, and two-spotted spider mite populations. The simultaneous introduction of both mites based on the availability of their preferred food was promising or possible. Under the same greenhouse conditions, the sole application of *B. pseudobassiana* on tomato provided the highest control against *T. absoluta* and leafminer (*Liriomyza spp.*) to below ETL followed by the microbial agent *Bacillus thuringiensis* and then chemical pesticides.

### Recommendations:

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

- i. Expand the rearing production units, for large scale production of natural enemies.
- ii. Collect and evaluate other potential biological control agents for the control of various arthropod pests, i.e.: *Macrolophus pygmaeus* and *Nesidicoris tenuis*.
- iii. Extend the research to efficacy of *P. persimilis* for the control of spider mites on greenhouse tomato in Lebanon.
- iv. Assess the thermal limit of the local strains of *A. swirskii* and *P. persimilis* for the possibility of thermotolerant strains
- v. Encourage registration and application of selective pesticides to reduce disease infections and high pest infestations without adversely affecting predator populations



- vi. Recommend to the Ministry of agriculture to initiate a fast track for registration of selective, safe pesticides.
- 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 and establishment of the mite population
- 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
- xi. Provide adequate training to extension officers and farmers on the use of natural enemies: Demonstration plots, workshops, farmer field schools, videos, pamphlets...

The preliminary results of the biologically based IPM are very promising and it is highly recommended to intensify these studies for the safety of humans, wildlife and the environment. The biodiversity in Lebanon provides a suitable niche for several natural enemies that could be collected and evaluated for their efficacy in pest control. While the adoption of alternative measures will require additional inputs and time, it will provide long-term benefits for the farmer, consumer and the economy of the country such as low pesticide residue levels, thus providing the possibility of exporting produce to the European Union and elsewhere at higher prices. The local production and integration of natural enemies for pest control in Lebanon will create

a significant long-term advancement in the agricultural sector, the country economy,  
food safety and food security

## APPENDIX I PESTICIDE AND BIO CONTROL AGENTS APPLICATIONS

Table 1 Dates of introduction and release rates of biocontrol agents throughout the growing period in IPM greenhouses in experiment 1 and 2.

	Experiment 1			Experiment 2	
	Greenhouse			Greenhouse	
	IPM-A	IPM-B		IPM-A	IPM-B
Date	Sprays	Natural enemy / Release Rate	Date	Sprays	Natural enemy / Release Rate
9/25/2019		<i>A. swirskii</i> (25/m <sup>2</sup> )	1/28/2020	-	-
10/2/2019	<i>B.pseudobassiana</i> (20L of 10 <sup>8</sup> spore/ml)	-	2/4/2020	-	-
10/9/2019	<i>B.pseudobassiana</i> (30L of 10 <sup>8</sup> spore/ml)	<i>A. swirskii</i> (25/m <sup>2</sup> )	2/11/2020	-	-
		<i>P.persimilis</i> (1000 mites, 12/ m <sup>2</sup> )			
10/16/2019	<i>B.pseudobassiana</i> (30L of 10 <sup>8</sup> spore/ml)	<i>P.persimilis</i> (620 mites, 12/ m <sup>2</sup> )	2/18/2020	-	-
11/1/2019	<i>B.pseudobassiana</i> (80L of 10 <sup>8</sup> spore/ml)	<i>A. swirskii</i> (50/m <sup>2</sup> ) <i>P.persimilis</i> (620 )	2/25/2020	-	-
		<i>B.pseudobassiana</i> in hotspots			
11/6/2019	-	Coragen® 20 SC	3/3/2020	<i>B.thuringiensis</i> (60L)	<i>B.pseudobassiana</i> (60L)
		<i>B.pseudobassiana</i> in hotspots			
11/14/2019	<i>B.pseudobassiana</i> (75L of 10 <sup>8</sup> spore/ml)	<i>A. swirskii</i> (50/m <sup>2</sup> )	3/10/2020	<i>B.thuringiensis</i> (60L)	<i>B.pseudobassiana</i> (60L)
		<i>B.pseudobassiana</i> in hotspots			
11/20/2019	-	-	3/17/2020	<i>B.thuringiensis</i> (60L)	<i>B.pseudobassiana</i> (60L)
11/28/2019	-	<i>B.pseudobassiana</i> (75L of 10 <sup>8</sup> spore/ml)	3/24/2020	<i>B.thuringiensis</i> (60L)	<i>B.thuringiensis</i> (60L)
4/12/2019	-	<i>B.pseudobassiana</i> in hotspots(75L of 10 <sup>8</sup> spore/ml)	3/31/2020	<i>B.thuringiensis</i> (80L)	<i>B.pseudobassiana</i> (80L)
-	-	-	4/7/2020	-	-

Table 2 The applied pesticide program throughout the growing period in the control greenhouse in experiment 1 and 2.

	Experiment 1	Experiment 2	
	Control greenhouse		Control greenhouse
Date	Sprays	Date	Sprays
9/25/2019	Abamectin and Lambda-Cyhalothrin	1/28/2020	Emamectin benzoate (5%) Abamectin Acetamiprid (20%)
10/2/2019	Acetamiprid (20%)	2/4/2020	-
10/9/2019	Acetamiprid (20%)	2/11/2020	Emamectin benzoate (5%) Acetamiprid (20%)
			Abamectin Acetamiprid (20%)
10/16/2019	-	2/18/2020	Chlorfenapyr Emamectin benzoate (5%) Abamectin
11/1/2019	Acetamiprid (20%) and Abamectin	2/25/2020	Emamectin benzoate (5%) Abamectin Acetamiprid (20%)
11/6/2019	Acetamiprid (20%) and Abamectin	3/3/2020	-
11/14/2019	-	3/10/2020	Chlorfenapyr Emamectin benzoate (5%) Abamectin
11/20/2019	Abamectin and Lambda-Cyhalothrin	3/17/2020	Chlorfenapyr Emamectin benzoate (5%) Abamectin
11/28/2019	-	3/24/2020	Emamectin benzoate (5%) Abamectin Acetamiprid (20%)
4/12/2019	-	3/31/2020	Emamectin benzoate (5%) Abamectin Acetamiprid (20%)

-	-	<b>4/7/2020</b>	Chlorfenapyr Emamectin benzoate (5%) Abamectin
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## APPENDIX II

### TEMPERATURE AND RELATIVE HUMIDITY

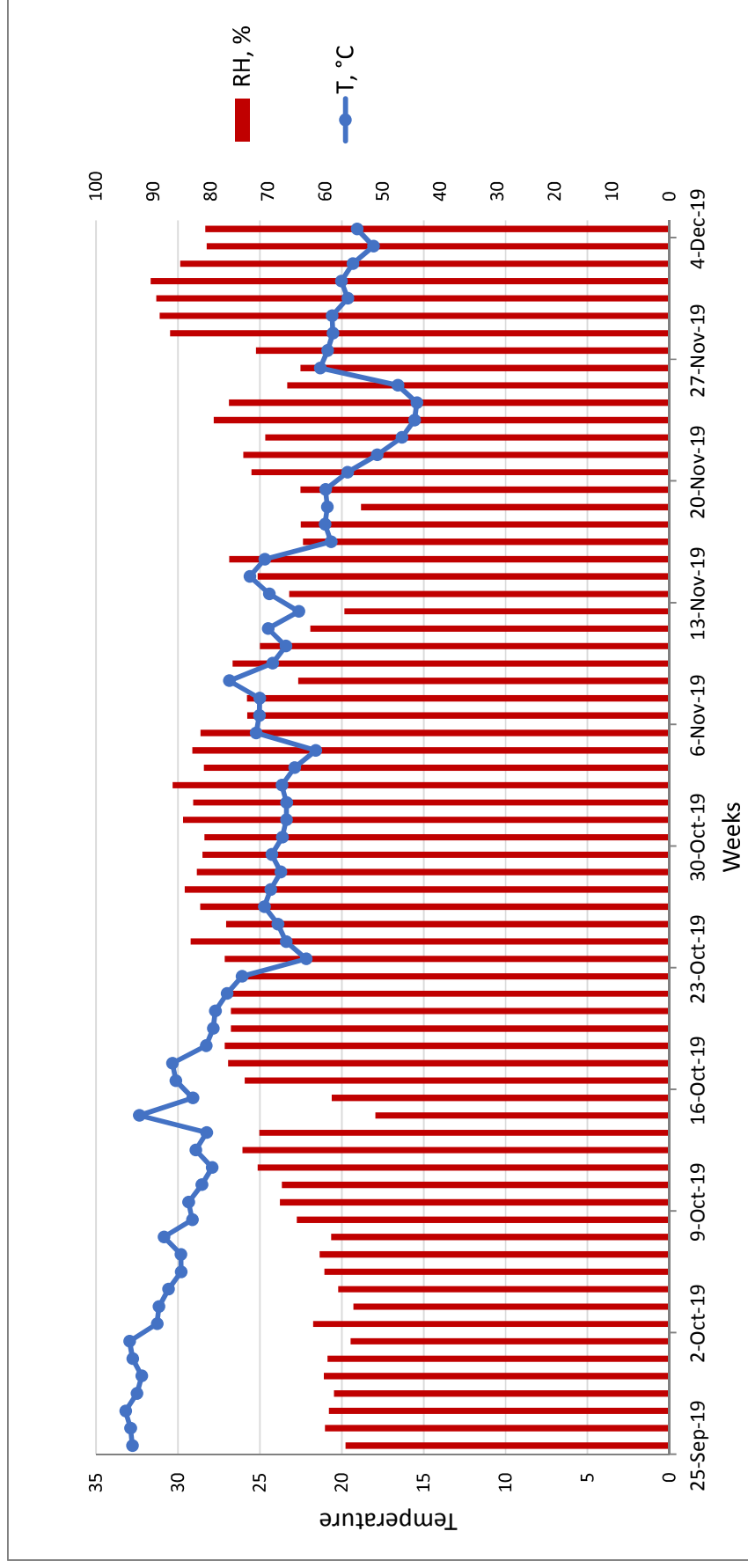


Figure 13. Average Temperature (°C) and RH (%) recorded throughout the growing period of Cucumber in Rmeileh

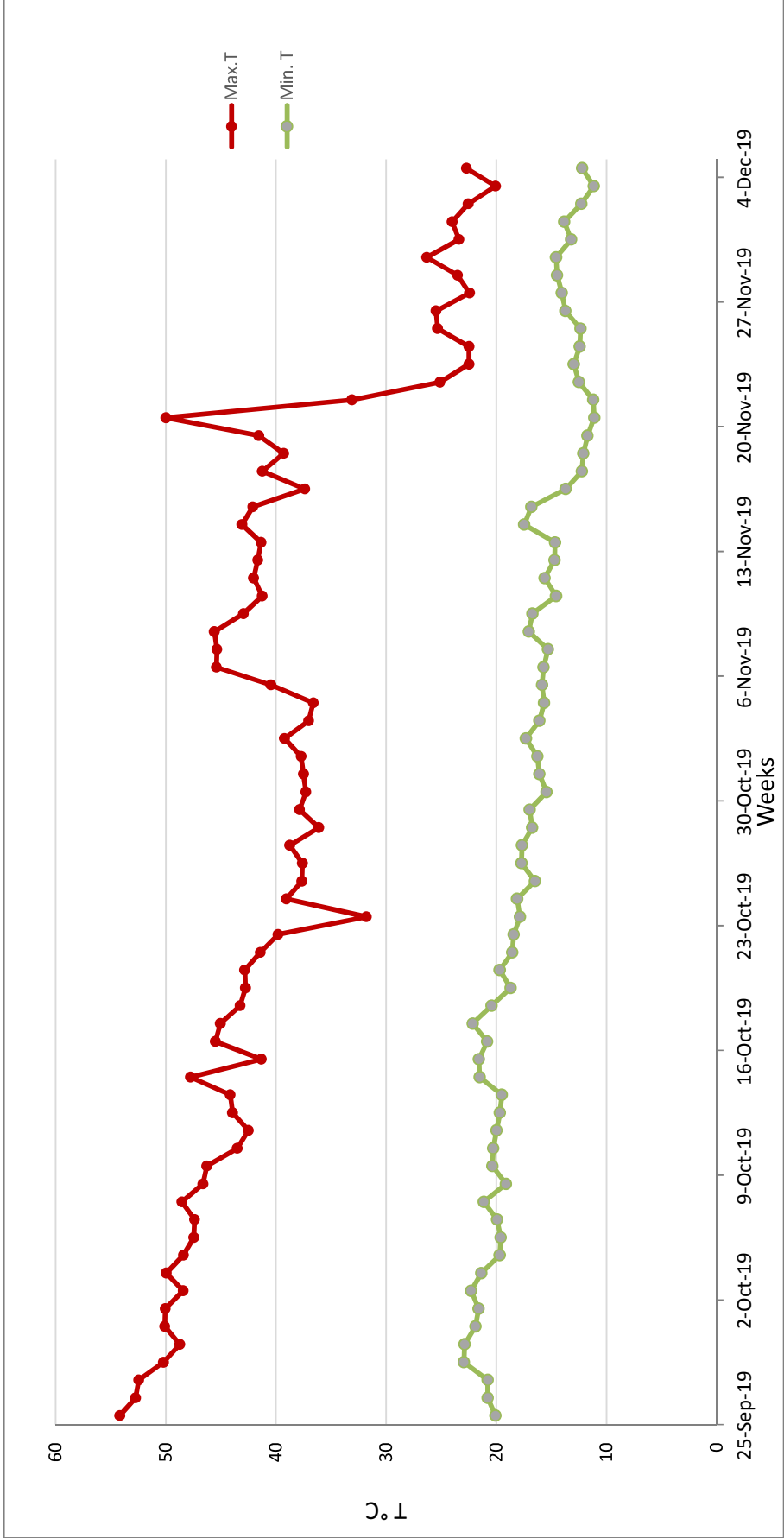


Figure 14. Maximum and Minimum Temperatures (°C) recorded throughout the growing period of Cucumber in Rmeileh.

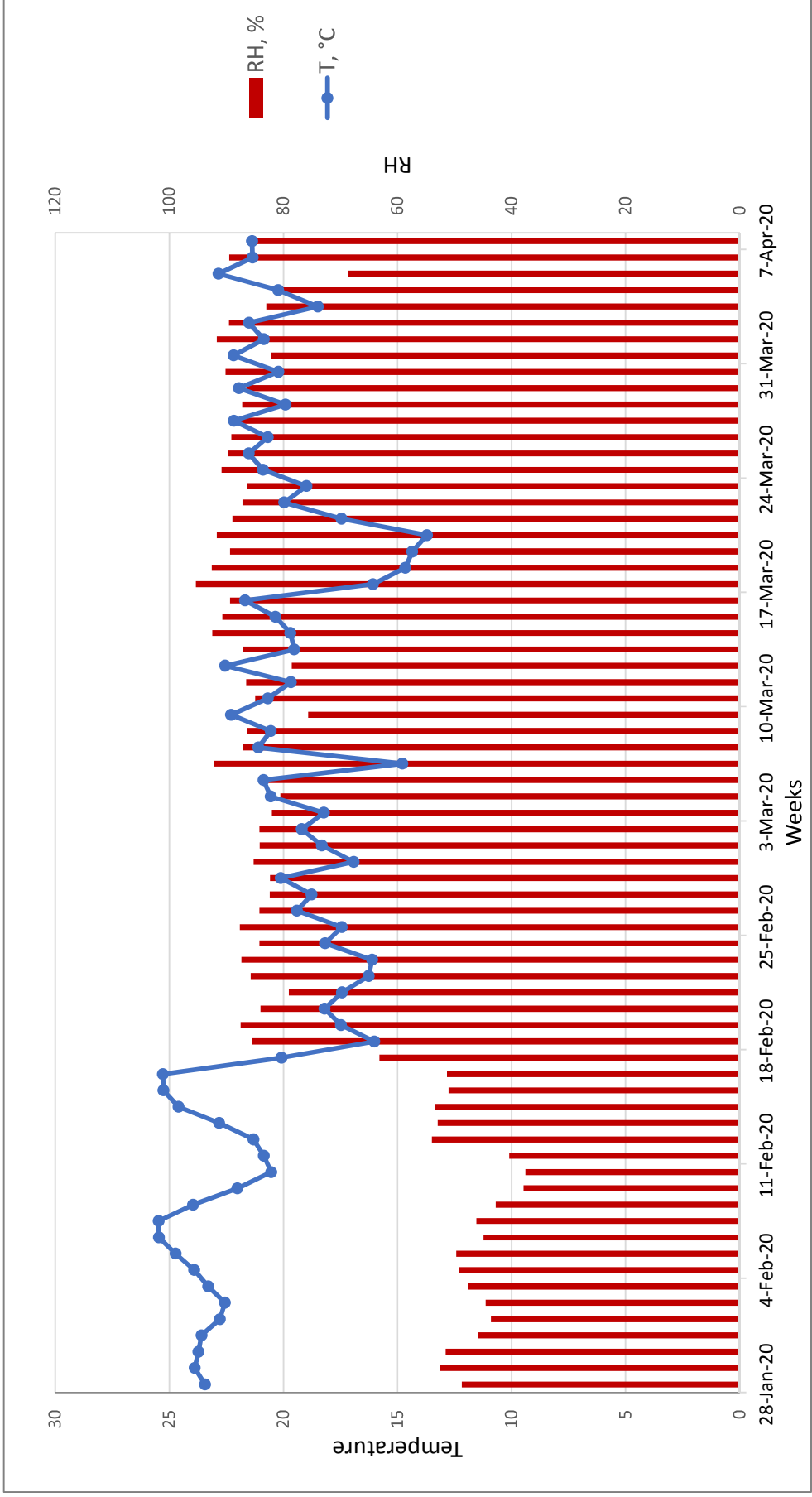


Figure 15. Average Temperature (°C) and RH (%) recorded throughout the growing period of Tomato in Rmeileh.



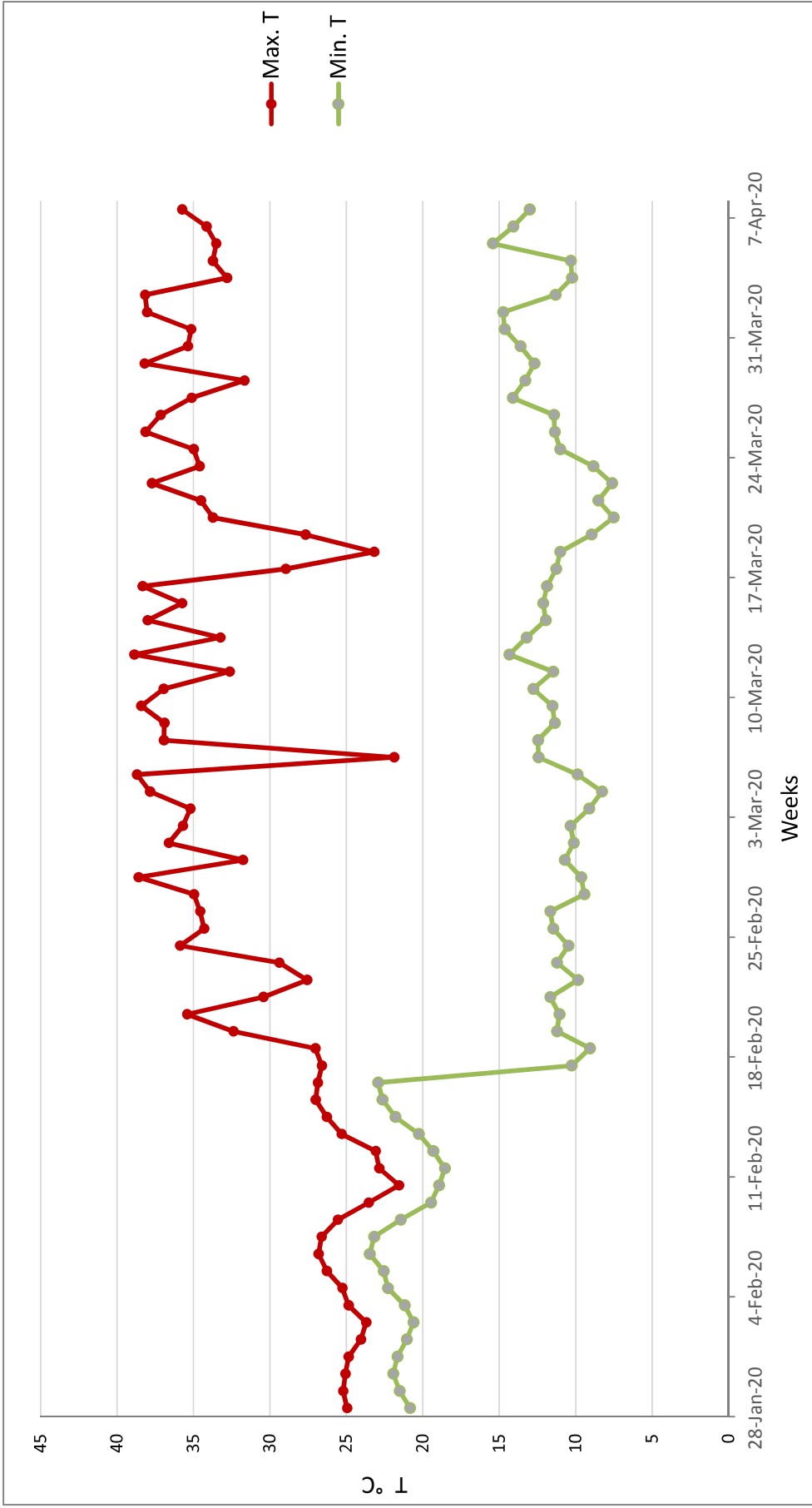


Figure 16. Maximum and Minimum Temperatures (°C) recorded throughout the growing period of Tomato in Rmeileh.

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