AMERICAN UNIVERSITY OF BEIRUT

INTEGRATED PEST MANAGEMENT OF GREENHOUSE PESTS BY LOCALLY COLLECTED ENTOMOPATHOGENIC FUNGI AND PREDATORY MITES

by

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AN ABSTRACT OF THE THESIS

Nour Issam Ezzeddine for Master of Science
Major: Plant Protection

Title: Integrated pest management of greenhouse pests by locally collected entomopathogenic fungi and predatory mites

An effective biological control based integrated pest management system has been developed for greenhouse Lebanese farmers, as an alternative to the use of pesticides. Pesticides have long been criticized for their harmful effects to the environment, plants, and human health and their misuse lead to a reduced efficacy in the control of pests due to buildup of pest resistance against many pesticides belonging to different chemical families. For that reason, mass production of a locally collected entomopathogenic fungus *Beauveria pseudobassiana* and the predatory mite *Phytoseiulus persimilis* were conducted and followed by laboratory and field studies to assess their efficacy in the control of arthropod pests. Mass production trials of *B. pseudobassiana* on 4 grain substrates at two grain: water ratios, showed that the highest yield was $3x10^9$ conidia g^{-1} harvested from burghul at 1:1 grain: water ratio. Laboratory tests then aiming at improving the germination percent of conidia showed that addition of mannitol at 10 g L^{-1} or 1% corn oil to the conidial suspension significantly improved conidia germination. The local fungal isolate was then tested for its efficacy in the control of *Myzus persicae* and *Bemisia tabaci* on cucumbers and *Tuta absoluta* on tomato, under greenhouse conditions. The treatments were 10^8 conidia $mL^{-1}$of *Beauveria* + 1% corn oil + 0.01% Tween-20 and 1% corn oil + 0.01% Tween-20. The fungus was able to reduce the *M. persicae* colonies by 83%, and the *B. tabaci* population by 88% compared to the control. On tomatoes, the sprays of *Beauveria* were effective in controlling the egg stage of *T. absoluta*, causing 80% corrected mortality at 20℃ and 70% RH, and 100% mortality at 14℃ and 75% RH. In parallel, mass production system of *P. persimilis* was established by examining three different ratios of spider mites: *Phytoseiulus*. The 40:1 ratio was the fastest in producing high numbers of predatory mites. Two small scale greenhouse experiments were conducted to determine the efficacy of *Phytoseiulus* against *Tetranychus urticae* and its compatibility with *Beauveria* within an integrated pest management program on cucumbers. The results demonstrated that the local *Phytoseiulus* showed 96% efficacy against spider mites and was compatible with the fungal isolate when sprayed 10^8 conidia mL^{-1}+ 1% corn oil + 0.01% Tween-20, resulting in 100% control. The local isolate of *B. pseudobassiana* was also highly active with 88% efficacy, without significant difference between the last three treatments. The biological agents were then evaluated in two commercial greenhouses cultivated with cucumber and pepper plants. In the control greenhouse, the farmer followed his normal plant protection practices and applied 14 sprays of pesticide mixtures but failed to prevent the outbreak of spider mites during June. While in the Integrated pest management (IPM) greenhouse, IPM practices were implemented including natural enemy releases. In the IPM

greenhouses, *P.persimilis* was highly effective in reducing spider-mite infestations, even at elevated temperature (T. average 26 ºC and max 40) recorded in May and June. In addition, three sprays of *B. pseudobassiana* were able to manage the population of aphids on pepper plants. Thus under commercial greenhouse conditions, it was possible to control all cucumber and pepper arthropod pests in Kfarmashoun area without using any insecticide or acaricide by relying on IPM practices and using three local natural enemies, *P. persimilis*, *B. pseudobassiana* and *Amblyseius swirskii* (data presented in another study). The extra costs incurred by using natural enemies may be compensated by a higher price of the better-quality fruits suitable for export. Local production of natural enemies may reduce their cost.

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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^2	Square meter
A. colemani	*Aphidius colemani*
A. gossypii	*Aphis gossypii*
a.i.	Active ingredient
ANOVA	Analysis Of Variance
A. swirskii	*Amblyseius swirskii*
B. bassiana	*Beauveria bassiana*
B. pseudobassiana	*Beauveria pseudobassiana*
BSC	Blue sticky cards
B. tabaci	*Bemisia tabaci*
B. thuringiensis	*Bacillus thuringiensis*
CABYV	*Cucurbit aphid-borne yellows virus*
CM	Corrected mortality
CMV	*Cucumber mosaic virus*

C. tannourinensis	*Cephalcia tannourinensis*
EIL	Economic injury level
et al.	*et alia* (and others)
ETL	Economic threshold level
FAO	Food and Agriculture Organization
Ha	Hectare
Hrs	Hours
IPM	Integrated Pest Management
L:D	Light to Dark ratio
LMV	*Lettuce mosaic virus*
M	Million
MD	Moderately toxic
M. persicae	*Myzus persicae*
NT	Not toxic
PDA	Potato Dextrose Agar
P. persimilis	*Phytoseiulus persimilis*
PRSV	*Papaya ringspot virus*
PVY	*Potato virus Y*
®	Registered trade mark
r	Intreinsic rate of increase
Spp.	Species
ST	Slightly toxic
S. tuberosum	*Solanum tuberosum*

T. absoluta	*Tuta absoluta*
T. urticae	*Tetranychus urticae*
T. vaporariorum	*Trialeurodes vaporariorum*
WMV	*Watermelon mosaic virus*
WP	Wettable powder
YSCs:	Yellow sticky cards
ZYMV	*Zucchini yellow mosaic virus*

CHAPTER I

INTRODUCTION

Over the next 20 years, crop production will have to increase by 70% to meet the rising global population and changing diets in developing countries (FAO, 2009; Ray et al., 2013). Crop productivity can be increased in different means such as by high-yielding varieties, improved water and soil management, fertilization, and other cultivation techniques. An increased yield potential, however, attracts more pests and diseases, often leading to an increase in total losses and loss rates (Cerda et al., 2017). Out of the several known arthropod pests causing damage on vegetables, whiteflies (*Trialeurodes vaporariorum* Westwood and *Bemisia tabaci* Gennadius), thrips (*Thrips tabaci* Lindeman and *Frankliniella occidentalis* Pergande), two-spotted red mites (*Tetranychus urticae* Koch), and aphids (*Aphis gossypi* Glover and *Myzus persicae* Sulzer) are namely the primary and most devastating arthropod pests on cucumber and tomato plants; (Wakil et al., 2018; Şovărel et al., 2019). In addition to the previously listed pests, *Tuta absoluta* Meyrick has been introduced recently to Lebanon and has been reported as a destructive gelechiid moth on tomatoes.

Pests, pathogens, and weeds are considered as a significant challenge for agricultural production, resulting in a potential yield loss of 90–100% of field-produced tomatoes in case no control measures were undertaken (Biondi et al., 2018). This shows the importance of crop protection in promoting food security and economic independence for the farmers (FAO, 2009). The conventional methods used in crop protection led to an

increase in the intensity of pesticide use by 15–20 folds worldwide without the alternation of pesticides with different modes of action (Oerke, 2005). Therefore, the development of insect resistance against most of the commonly applied insecticides was a serious issue facing agriculture (Desneux et al., 2010; Guedes & Picanco, 2012). In parallel, the maximum residue levels (MRLs) of pesticides in crops are recorded in higher doses than before, and the rational use of these synthetic chemicals is sharply linked to biodiversity loss in the agricultural habitats. Thus, alternative control methods must be implemented to maintain pest populations below their economic threshold and avoid crop damage. The environmentally safe alternative solution to the use of synthetic chemicals is the application of biological control agents within an integrated pest management program (IPM). IPM has been recognized as an effective strategy to manage the pest population, mainly through the integration of several environmentally-friendly control practices (Parsa et al., 2014), while minimizing the potential risk of development of insect/mite resistance by alternating between cultural, biological, mechanical/physical, and chemical methods.

The objectives of this study are:

1. Mass production of the Lebanese entomopathogenic fungal isolate *Beauveria pseudobassiana* and evaluating its efficacy in the control of whiteflies, aphids, and spider mites under laboratory and normal greenhouse production conditions.
2. Mass rearing of locally collected *Phytoseiulus persimilis*, a predatory mite on spider mites *Tetranychus urticae* and assessing its efficacy under standard greenhouse conditions.
3. Determine the compatibility of *B. pseudobassiana* and *P. persimilis* in an IPM program.

CHAPTER II

LITERATURE REVIEW

A. Protected cultivation

Among the productivity-enhancing technologies, better quality produce and protection from pests and diseases, protected cultivation has a tremendous potential over the open field (Talukdar et al. 2003). Greenhouse crop production is practiced throughout the world, with an estimated 405,000 ha of greenhouses spread over all the continents irrespective of the altitude (FAO, 2013). In Europe, Spain is leading in protected agriculture with 52,170 ha, mostly under low-cost polyhouses. In Asia, of greenhouse production areas totals a 224,974 ha, with China having the largest area under protected cultivation. Tomato, bell pepper, cucumber, rose, carnation, and gerbera are the most extensively grown vegetable and ornamental crops under greenhouses to achieve higher returns (Chandra et al. 2000).

B. Vegetable production in Lebanon

1. Cucumber

Cucumber (*Cucumis sativus* L.) is one of the most grown vegetable crops throughout the world (Soleimani et al., 2009). Cucumber is grown for its tender fruits, which are consumed either raw as a salad, cooked as a vegetable, or pickled in its immature stage. The calorific and nutritional value of cucumber is deficient, but it is rich in β-

carotene, Vitamin A and potassium (Abrahamian, 2014), and it is a primary source of minerals and fiber for the human body (Keopraparl, 1997). Cucumber is a thermophilic and frost susceptible crop; it grows successfully under conditions of high light intensity, high relative humidity, high soil moisture, elevated temperature range, and fertilizers in greenhouses (El-Aidy et al., 2007). The protected cultivation provides a favorable environment to cultivate cucumbers year-round, with the desirable qualities of fruits and high yields.

Moreover, the production of crops under greenhouse conditions favorized the use of biocontrol agents (Mahesh et al., 2017). During 2018, world cucumber and gherkins production reached around 83 M tons with a cultivated area of 2 M Ha, with China being the lead producing country. In Lebanon, total cucumber and gherkins production reached 167,037 tonnes on a cultivated area of 3,265 Ha (FAOSTAT, 2018). The production of cucumber in Lebanon accounts for 11% of the total grown vegetables (FAO, 2014).

2. Tomato

Tomato (*Solanum lycopersicum* L.) belongs to the nightshade family, known as Solanaceae. It is the most cultivated vegetable worldwide. Solanaceae family encompasses many other important crops like potato (*Solanum tuberosum* L.), pepper (*Capsicum spp.),* eggplant (*Solanum melongena* L.), and tobacco (*Nicotiana tabacum* L.) (Bosland, 1992; Heuvelink, 2005). Tomatoes are perennial vegetables in their native habitat, but due to their sensitivity to low temperatures, they are grown as annual plants in temperate regions (Heuvelink, 2005; Peet & Welles, 2005), growing best at temperatures between 20–27°C. They are moderately salt tolerant; however, high salt concentrations may cause a significant

reduction in tomato fruit quality and yield, thus leading to considerable economic losses (Heuvelink, 2005; Li, 2000). While most often associated with lycopene, tomatoes provide a unique variety of phytonutrients such as carotenoids, flavonoids, hydroxycinnamic acids, glycosides, and fatty acid derivatives. Tomatoes are also an excellent source of free radical-scavenging vitamin C and vitamin A, as well as bone-healthy vitamin K (Ganesan et al., 2012). The total production of tomato worldwide has reached 200 M tonnes on a cultivated area of 4.8 M Ha during 2018. In Lebanon, tomato is grown on a total harvested area of 3,810 Ha, producing 330,822 tonnes (FAOSTAT, 2018), as it ranks first among greenhouse grown vegetables.

3. Pepper

Pepper (*Capsicum annum* L.) belongs to the family Solanaceae, which is an economically major group of vegetables grown worldwide (Channabasavanna, 2000). It grows best in a warm climate and requires temperatures ranging between 25-35°C, where frost is not a problem during the growing seasons (Olalla and Valero, 1994). Sweet pepper, also known as Bell pepper, is the world's second most important vegetable after tomato (Anonymous,1989). Sweet pepper contributes substantially to our diet; it is a good source of vitamins A, C, E, B1, B2, and D (Muhamman and Auwalu, 2009). Globally, 744,278 metric tons of pepper were produced, where Asia produces more than 50% of the world's pepper, a total of 549,595 metric tons, whereas the United States (U.S.) produced about 118,136 metric tons (FAFOSTAT, 2018). In Lebanon, chilies and pepper are grown on a total harvest area of 604 Ha, producing 26,310 tonnes (FAOSTAT, 2018).

C. Greenhouse arthropod pests in Lebanon

Greenhouses provide a plethora of food coupled with environmental conditions that favor pest population development over time. Out of the most economically important pests found in Lebanon, whiteflies, aphids, tomato pinworm and spider mites are considered the most damaging pests.

1. Whiteflies

a. Classification

The Aleyrodidae family includes around 120 genera and 1,300 to 1,500 species of 1-3 mm long insects known as whiteflies in the order Hemiptera (Jones, 2003; Navas-Castillo et al., 2011). The main pests of this family infesting cucumber greenhouses are the greenhouse whitefly known as *Trialeurodes vaporariorum* Westwood and the sweet potato whitefly known as *Bemesia tabaci* Gennadius (Hemiptera: Aleyrodidae), the latter being prevalent in Lebanon and in relatively hot climates.

b. Morphology

B. tabaci is major pest on agricultural crops in the Middle East, North and Central America, Europe, and the Caribbean basin (Salas & Mendoza, 1995). It is characterized by its fourth-instar nymphs, they have no waxy filaments or marginal fringe and the adults have white wings and yellow bodies; they hold their wings in a tent-like form above their body (Gill, 1990). However, *T. vaporariorum* is characterized by very long waxy filaments and marginal fringe on the fourth-instar nymphs, in addition to the adults that have white

wings and a yellow surface or substrate situated in a triangular form above the abdomen (Fig. 1).

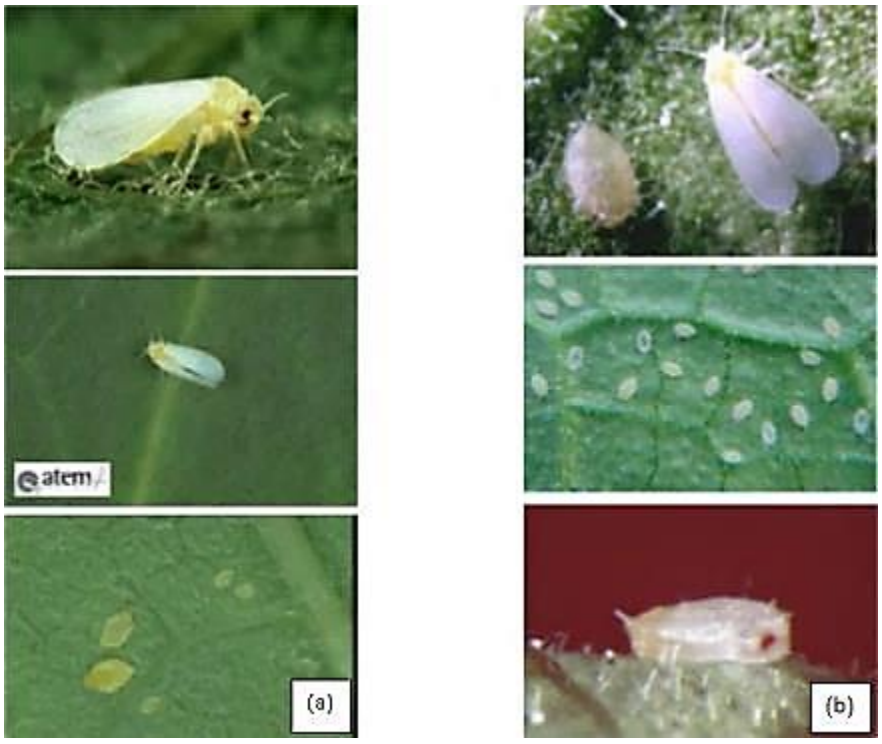

Figure 1.Morphology of different life stages of *B. tabaci* (a) and *T.vaporariorum*(b) (Organic. Vegetation, 2013).

c. Lifecycle

Females of *B. tabaci* lay white eggs in circular patterns on the undersides of leaves. Each female can lay up to 200 eggs during her lifespan. At 25°C, the average development time of whiteflies from egg to adult stage is around 20 days on cucumber plants (Malais & Ravensberg, 2004). The eggs will hatch in 7 days and the mobile larvae move over the leaves for five days, then settles and molts to a sedentary 'scale' stage. One week later and

after the 2nd molt, the last stage larva remains where it was feeding and pupates (4th larval instar); adults then emerge. Many whitefly pest species are multivoltine, producing several generations a year. They also tend to develop resistance rather quickly to a large number of pesticides (Byrne et al., 1990).

d. Host range and damage

The sweet potato whitefly, *Bemisia tabaci*, is particularly abundant in warm habitats, and feeds on over 1000 plant species (Abd-Rabou et al., 2010). Whiteflies damage occurs through sucking plant sap, which weakens plants and causes shoot and leaf distortion. A more severe problem is the large amount of honeydew they secrete onto leaves and fruits. The honeydew is then colonized by sooty molds, reducing the quality of greenhouse vegetables and ornamentals (Navas-Castillo et al., 2011). Whitefly pest species are of greater economic importance as vectors of plant viruses; they can transmit Geminiviruses, Carlaviruses, Nepoviruses, Potyviruses and Closteroviruses (Navas-Castillo et al., 2011, Luan et al., 2011). The economic threshold for whiteflies is 5 adults per cucumber leaf (Jeon et al., 2009).

e. Pest management

The use of insecticides was the first approach employed to manage whitefly populations, and the control of whiteflies should start when there are 5 adults per cucumber leaf (Jeon et al., 2009; Shen et al., 2005).The most extensively used insecticide classes were the organochlorines, organophosphates (OPs), carbamates, and pyrethroids (Palumbo et al., 2001). During the late 90s, the OPs and pyrethroids have been replaced by neonicotinoids

and other compounds of novel chemistry, worldwide (Jeschke et al., 2010). *B. tabaci* was able to develop resistance to spiromesifen and spirotetramat, a new class of insecticides interfering with lipid biosynthesis, characterized as a new mode of action (Bielza et al., 2019).This practice of relying on insecticides has been greatly restricted; however, due to both environmental concerns and the widespread resistance that has developed to most of the insecticides in use (Horowitz et al., 2007; Castle et al., 2010, Whalon et al., 2013). Consequently, increasing importance is being placed upon control by other methods (including cultural, mechanical, and biological) as a means of managing pest populations.

2. Aphids

a. Classification

Aphids, as a group, represent some of the most damaging insects to agriculture in the world. Of the approximately 4700 species in the family Aphididae, nearly 100 are critical agricultural pests (Silva et al., 2012). In protected cultivation, the most economically important species attacking vegetable crops are *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas, *Aulacorthum solani* Kaltenbach, and *Myzus persicae* Sulzer (Thrope et al., 2016).

b. Morphology

M. persicae is a highly variable species; strains, races, and biotypes have been distinguished based on morphology, color, biology, host-plant preference, ability to transmit viruses and insecticide resistance (Bass et al., 2015). The eggs measure about 0.6 mm long and 0.3 mm wide and are elliptical. Eggs initially are yellow or green, but

soon turn black. Mortality in the egg stage might be quite high. Instars are initially greenish, but soon turn yellowish, much resembling viviparous (parthenogenetic, instar-producing) adults. Adult wingless parthenogenetic females are oval-bodied, 1.2–2.1 mm in body length, and can vary in color from whitish-green, pale yellow-green, gray-green, mid-green, dark green, to pink or red (Blackman and Eastop, 2007). Winged (alate) aphids have a black head and thorax, and a yellowish green abdomen with a large dark patch dorsally (Fig. 2). They measure 1.8 to 2.1 mm in length. Winged green peach aphids seemingly attempt to colonize nearly all plants available.

Figure 2. *M. persicae* winged adult and instar stages (Koppert®)

c. Life cycle

The life cycles of aphids are among the most remarkable ones; they include both the parthenogenetic and sexual generations (Rousselin et al., 2017). During the growing season

and in greenhouses, aphids continuously give birth to live young (viviparous reproduction) without mating. Winged aphids develop when aphid populations are high or during spring and fall seasons. On outdoor plants, during fall and in response to short daylength, male aphids develop in colonies; they mate with the females, which then produce eggs. The eggs overwinter on perennial plants and hatch in the spring. These fly to new plants and can be transported very long distances in air currents to infest a crop quickly (Flint, 2013).

d. Host range and damage

These pests can have a dramatic impact on the production of vegetables, fruits, and ornamentals (Riddick, 2017). They colonize the Solanaceae, especially potato and tomato, and plants in the Cucurbitaceae, Chenopodiaceae, Compositae, Leguminosae, Rosaceae in addition to numerous ornamental plant species (Mardani-Talaee et al., 2016). Aphids damage plants in three significant ways. First, aphids suck plant sap, which causes distortion of enlarging leaves and shoots and reduces the vigor of plants. Heavy infestations can result in plant death. Second, as they feed, aphids produce sticky, sugary substrate, the 'honeydew", which provides a suitable medium for the growth of black sooty mold fungi (from the genera *Capnodium*, *Fumago*, *Scorias*, *Antennariella*, *Aureobasidium*, and *Limacinula*). The third, and perhaps most costly type of aphid damage, is that aphids are efficient vectors of several plant viruses (Quisenberry & Ni, 2007; Katis et al., 2007). They can transmit *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Lettuce mosaic virus* (LMV), *Papaya ringspot virus* (PRSV) (Dedryver et al., 2010), *Zucchini yellow mosaic virus* (ZWMV), *Watermelon mosaic virus* (WMV) (Perring et al., 1992), and *Cucurbit aphid-borne yellows virus* (CABYV) (Carmo-Sousa et al., 2016).

e. Pest management

The first means of control were pesticides which were fast-acting, advantageous, easy for the grower to apply, and were broad-spectrum, killing many pest insects with single applications. Organochlorines, organophosphates, carbamates, formamadines, and pyrethroids were or have been used for insect and aphid control (Philippou et al., 2010). While these pesticides provided outstanding results for killing insects, they also had adverse effects on the environment and natural enemies. Several other chemical groups are registered for *M. persicae* control in some crops (e.g. tetramic acids, sulfoximines), neonicotinoids are often considered to be the 'final frontier' of insecticide control for *M. persicae* within Australia. As well as being registered widely as a foliar spray, neonicotinoids are commonly used in grain crops as a seed coating prior to sowing and are applied through drip irrigation lines in many vegetable crops. Neonicotinoids such as thiamethoxam, clothianidin and acetamiprid are mostly unaffected by the mechanisms conferring resistance to organophosphates, carbamates and pyrethroids, although imidacloprid has been shown to be partially affected by enhanced expression of *E4*/*FE4* esterase (Philippou et al., 2010). However, specific resistance mechanisms to neonicotinoids in *M. persicae* have been identified worldwide. Resistance was first reported in Greece in 2007 (Philippou et al., 2010; Puinean et al., 2010) and over the last 9 years it has emerged in other European countries and the United States. Therefore, novel means of control that are effective and ecofriendly are required for the management of aphids.

The decision to apply control measures for aphids is based mainly on visual counts; measurable thresholds (economic injury level and economic threshold) have not been

determined on most of the vegetables. It is important to treat early symptoms to ensure that the aphids do not build up to high levels. However, it has been estimated that 50 aphids on any leaf in the upper third of the plant is the current economic threshold for the southeastern United States, on tobacco plant (*Nicotinana tabacum*, L). Plants below the 50 aphid threshold were considered uninfested (Merchán & Burrack, 2017).

3. Two-spotted spider mites

a. Classification

Mites are a diverse group of arthropods belongs to the class Arachnida and the subclass Acari and are among the most damaging pests worldwide (Merchán & Burrack, 2017). Spider mites (family: Tetranychidae) are especially troublesome, as an outbreak of these pests can lead to severe losses and even total failure of the cucumber crop. Within the spider mite family, the most widespread and abundant cucumber pest is *Tetranychus urticae* Koch (Acari: Tetranychidae), known as the two-spotted spider mite (TSSM).

b. Morphology

T. urticae eggs are about 0.15 mm in diameter, round, and translucent when first laid but become white as hatching approaches. Most often, the eggs are laid on the underside of leaves. Immature mites have the same general color and shape as adults, although the larvae have only 6 legs. A few days later, the nymphs will develop into 8-legged stage of protonymph and deutonymphs within 4-5 days before reaching the adult stage. The adult female is 0.6 mm long (Fig. 3), pale green or greenish-yellow (Boudreaux

and Dosse, 1963, English-Loeb, 1990) with two darker patches on the body (Shih et al., 1976; English-Loeb, 1990), which are oval with 12- long hairs on the dorsal side. Overwintering females are orange-red. On the other hand, the male has a smaller, narrower, and a more pointed body than the female (Fasulo et al., 2009).

Figure 3. Two females and one egg of the two-spotted spider mite (Petegem et al., 2016).

c. Life cycle

The life cycle and developmental stages of the spider mites are rapid, particularly at high temperatures, the optimum temperature for development is 25-27°C (Table 1). The life cycle of spider mites is composed of 5 stages, eggs, larval/nymphal, protonymph, deutonymph and the adult stage (Fasulo et al., 2009).

T. urticae can produce high population densities within a brief period due to their short developmental time and high fecundity rate (Danks 2006; Kavousi et al., 2009). Each female can lay an average of 90-110 eggs during a lifetime of about 30 days, with a pre-

oviposition period of 1-2 days (Zhang, 2003). Besides, *T. urticae* can reproduce not only through sexual reproduction but also, through arrhenotoky when necessary (Arrhenotokous reproduction enables a single virgin female mite to initiate a normal bisexual population that can potentially lead to significant economic loss) (Toyoshima & Amano, 1999).

T. urticae overwinters in the form of an adult female that is initiated by decreased temperature, short photoperiod and scarce food supply. The overwintering females stop egg-laying and feeding, and they hibernate in cracks and crevices in protected places, such as the soil or glasshouse structures. The females then resume activity in the spring when they lay eggs on leaves (Ghazy et al., 2016).

Table 1. Duration and survival of *T. urticae* and *P. persimilis* at different seasons (Hoque, 2008)

Stage	*Tetranychus urticae*		*Phystseiulus persimilis*	
	Duration	Survival (%)	Duration	Survival (%)
Summer				
Egg	3-4	100.00	1-2	100.00
Larva	4-5	48.2 ± 2.60	2-3	66.3 ± 2.45
Protonymph	5-6	33.4 ± 2.40	3-4	50.6 ± 3.16
Deutonymph	6-7	23.6 ± 1.28	4-5	40.5 ± 3.16
Adult	7-8	20.0 ± 1.02	5-6	32.0 ± 3.74
Autumn				
Egg	3-5	100.00	2-3	100.00
Larva	5-7	53.7 ± 2.21	4-5	78.5 ± 2.00
Protonymph	7-8	39.1 ± 1.61	5-6	38.4 ± 5.10
Deutonymph	8-9	32.2 ± 1.92	6-7	48.0 ± 3.74
Adult	9-10	28.4 ± 1.79	8-9	40.2 ± 4.47
Winter				
Egg	7-9	100.00	4-5	100.00
Larva	9-12	43.1 ± 1.64	6-8	86.4 ± 2.45
Protonymph	13-15	29.6 ± 1.33	9-10	60.1 ± 3.16
Deutonymph	15-17	21.3 ± 0.99	10-11	54.3 ± 4.00
Adult	17-19	16.2 ± 1.15	12-14	48.6 ± 2.00

d. Host range and damage

A wide variety of host plants (>11,745) have been recorded in 5,380 different geographical localities (Migeon et al., 2010). Spider mites can cause high economic losses to vegetables, ornamentals and trees (Attia et al., 2013). A decrease in the overall crop production, decline and death of the host plants are due to the feeding habit of spider mites on the leaf chlorophyll (Tehri et al., 2014; Pascual-Ruiz et al., 2014). When food resources become depleted, *T. urticae* are characterized by a unique dispersal mechanism of ballooning and crawling, facilitate their movement to new host plants, disperse over long distances, and to colonize widely separated plants (Bell et al., 2005). The ballooning spider

mites often accumulate to form a "silk ball", which is composed of immature females and disperse by the aid of wind (Clotuche et al., 2011, 2013).

e. Pest management

The primary means of mite control in the agricultural and medical fields is by applying acaricides. However, the intensive reliance on conventional acaricides has led to the build-up of resistance against these active ingredients (Van Leeuwen et al., 2010; Attia et al., 2013). This phenomenon is due to the short life cycles of mites, proliferous progeny and the sexual/asexual type of reproduction and biology of mites. Accumulation of resistance alleles throughout the generations and cross-resistance to new acaricides is hypothesized (Van Leeuwen et al., 2010; Bi et al., 2016). Control against spider mites should start at 2 spider mites per leaf. In these circumstances, the conventional methods of mite control should be substituted by integrated pest management strategies and chemical control should include the alternation of active ingredients with new modes of action.

4. Tomato pinworm, Tuta absoluta

a. Classification

The species was first named as *Phthorimaea absoluta* by Meyrick in 1917 and then redescribed as *Tuta absoluta* in 1994 (EPPO, 2005). It is a Lepidoptera and belongs to the Gelechiidae family, a taxon encompassing >4,000 species and including critical pests, notably Neotropical ones. Being well established as an important tomato pest in South America for more than 50 years, *T. absoluta* was first introduced in Spain in 2006 (Biondi

et al., 2018; EPPO 2019) and then rapidly spread across the Mediterranean coastal tomato-producing areas (Desneux et al., 2010, Desneux et al., 2011).

b. Morphology

The eggs of *T. absoluta* are small (0.36 x 0.22mm), cylindrical and creamy white to yellow or brownish (Calvo et al., 2012). The eggs will then hatch into cream-colored larvae with a dark head, becoming greenish to light pink in the second to fourth instars. Then the brownish pupae may be found in or on the leaves or the soil, and occasionally on the flowers, fruits and growing points. The adult will eventually emerge, they are 10 mm long, with filiform antennae, silverish-grey scales, black spots on anterior wings (Biondi et al., 2018) (Fig. 4).

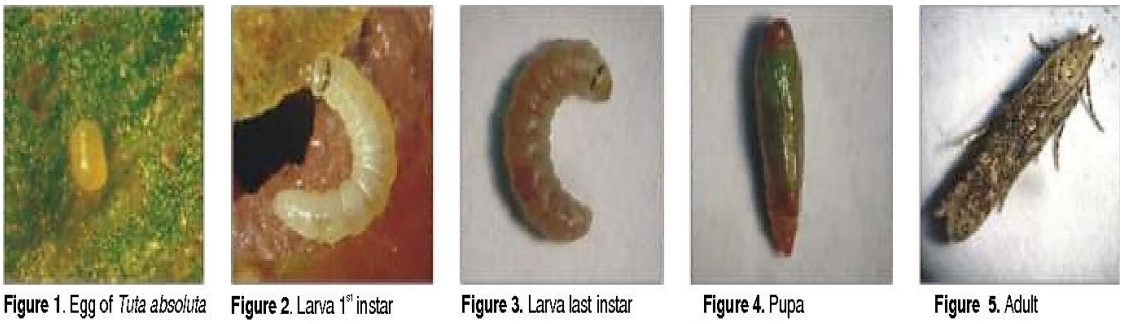

Figure 4. *T. absoluta* life stages.1. Egg; 2. 1st instar larva; 3. last instar larva; 4. Pupa; 5. Adult (Harizanova et al., 2009).

c. Life cycle

Eggs are laid individually by females 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 by mining below sepals (Desneux et al., 2010). Mature larvae usually drop to the soil where they produce a thin, silky cocoon to transform into prepupae and pupae. Adults are crepuscular, and both genders are sexually active by the first day of emergence. Mating communication relies on female sexual pheromones, and mating lasts from a few minutes up to six hours (Lee et al., 2014); females can produce ≤260 eggs during a lifetime (Uchoa-Fernandes et al., 1995).

The optimal temperature for *T. absoluta* development is 30℃, and life cycle duration varies from 26 to 75 days, with upper and lower developmental thresholds estimated at 34.6 and 14◦C, respectively (Martins et al., 2016). These biological characteristics enable *T. absoluta* to undergo up to 10 generations per year and to survive during cold seasons in protected and open-field crops (Cocco et al., 2015).

d. Host range and damage

Vegetables of the Solanaceae family like tomato, potato, and European black nightshade (*Solanum nigrum*) are the primary host plants of *T. absoluta* (Desneux et al., 2010). However, it can oviposit and develop on multiple plants belonging to the Amaranthaceae, Convolvulaceae, Fabaceae, and Malvaceae families (Bawin et al., 2016). Females use plant volatiles for orientation toward host crops, and leaf contact is a crucial component for inducing oviposition (Proffit et al., 2011).

Without adequate controls, infestations of *T. absoluta* can result in a 90–100% loss of field-produced tomatoes (Biondi et al., 2018; Marchioro et al., 2017). *T. absoluta* is a major limiting factor for tomato production in South America (Luna et al., 2012). Plant injury consists of mine-formation within the mesophyll by feeding larvae, thus affecting the

plant's photosynthetic capacity, and resulting in lower fruit yield (Desneux et al., 2010). Heavy infestations can lead to complete defoliation of the plant. The most significant losses occur from feeding activity on the fruit (Biondi et al., 2018), which can further be colonized by pathogens causing fruit rot.

e. Pest management

Early complaints regarding efficacy of insecticides used against *T. absoluta* in Chile led to concerns about insecticide resistance (Guedes et al., 2012). In South America, low levels of pyrethroid resistance, followed by further detection of resistance to abamectin, cartap, and the organophosphate methamidophos, were observed (Salazar & Araya, 2001). While a widespread pyrethroid resistance was detected in Europe (Silva et al., 2011), and moderate resistance to indoxacarb, spinosad was detected in Europe and South America (Campos et al., 2015; Guedes et al., 2012). It promoted a notable increase in use of chitin synthesis inhibitors, leading to high levels of resistance to most of these compounds (Gontijo et al., 2013; Silva et al., 2011). Nevertheless, resistance to diamides, a relatively new class of insecticides, is rising in Brazil and Europe (Campos et al.; 2015; Roditakis et al., 2015). Curiously, reports on chlorfenapyr resistance in *T. absoluta* populations are still sparse (Silva et al., 2011). Insecticide resistance may lead to control failure, justifying the attention paid to this phenomenon (Guedes et al., 2017); however, estimating the likelihood of such failure requires suitable methods. Microbial control has been evaluated and relies mostly on commercial strains of *Bacillus thuringiensis* (*Bt*) var. *kurstaki* and *aizawaii* that kill larvae when ingested (González-Cabrera et al., 2011). Other *Bacillus* spp. as well as the fungi *B. bassiana* and *M. anisopliae* have been studied (Borgi et al., 2016; Contreras et al.,

2014) but have not resulted in commercial products specifically designed for *T. absoluta* yet.

D. Pest Management Decision-Making

Integrated pest management or IPM is defined as a holistic 'approach' or 'strategy' to combat plant pests using all available methods, with minimal applications of chemical pesticides (Dent, 2000; Peshin & Dhawan, 2009). The aim is not to eradicate pests, but to manage them, maintaining their populations below economically injurious levels (Smith et al., 1967). Putting this vision into practice would reduce not only farmers', consumers', and the environment's exposure to toxic compounds, but also problems caused by pesticide-resistant pests. A European Union (EU) Directive (European Parliament, 2009) has obliged all professional plant growers within the Union to apply the general principles of IPM since 2014. When setting up an IPM program, it is essential to start with preventing the pests from establishing in the field. This depends on applying mechanical, physical, and cultural methods before the growing season. The second step is to set an action threshold, the economic injury level and economic threshold. The economic injury level was defined by Stern et al. (1959) as the lowest population density that will cause economic damage. The economic threshold is defined as the pest population density at which control should be initiated to prevent an increasing pest population from exceeding the economic injury level (Stern et al., 1959; Pedigo et al., 1986). In parallel, it is critical to monitor and identify the pests, whether they are innocuous, beneficial or as pests, and their control will then be made depending on their action threshold. The final decision tool in IPM, will be the management, either with pheromones to disrupt pest mating, mass trapping, or application

of biological control and releasing natural enemies., If additional control methods are required, then targeted spraying of pesticides can be applied, and broadcast spraying of non-specific pesticides would be the last resort.

In this study the focus will be on the mass production of biological control agents and their application against vegetable arthropod pests.

E. Biological control

According to Dreistadt (2007), biological control is the beneficial action of predators, parasites, pathogens, and competitors in controlling pests and their damage. Biocontrol provided by these living organisms is especially important for reducing the numbers of pest insects and mites. Interest in biological control has increased over recent decades for many reasons (Van Lenteren et al., 2018). It promoted development of more sustainable farming practices, controlled pests that have built up resistance to most of the synthetic chemicals (Omkar, 2016), and fulfilled the consumers demand for pesticide free products (Van Lenteren et al., 2018).

Biological control agents known as natural enemies include predators, parasitoids and pathogens (Omkar, 2016). Predatory arthropods are beneficial because they feed directly on other insects like aphids, thrips, whiteflies, spidre mites. Common predatory arthropods include *Chrysoperla carnea* Stephens, *Macrolophus pygmaeus* Rambur, the predatory mites *Amblyseius swirskii* Athias-Henriot, and *Phytoseiulus persimilis* Evans. Example of a predatory insect commonly found in garden is *C. carnea* or the green lacewing. The adult primarily feeds on nectar and other fluids, but its larva is able to feed

on soft bodied insects like aphids. Furthermore, *A. swirskii* has attracted substantial interest as a biological control agent of mites, thrips and whiteflies in greenhouse and nursery crops (Bolckmans et al., 2005).

Parasites are usually much smaller than their hosts and have a shorter life cycle than their hosts. Parasitoids are frequently used for insects that parasitize other insects, parasitoids may comprise up to 25% of all insects (Parasitoids, Nick Mills, University of California, and Berkeley). Most parasitoids belong to the Hymenopterans or Dipterans orders. Parasitoids are able to attack different life stages of pest. Success stories include the release of *Eretmocerus eremicus* Rose and Zolnerowich, and *Encarsia formosa* Gahan against the nymph stages of whiteflies (Gerling, 1965).

Pathogens are microorganisms such as bacteria, fungi, nematodes, protozoa, and viruses that can infect and kill the host. Populations of some aphids, caterpillars, mites, and other invertebrate are sometimes drastically reduced by naturally occurring pathogens, usually under conditions such as prolonged high humidity or dense pest populations. In addition to naturally occurring disease outbreaks, some beneficial pathogens are commercially available as biological or microbial pesticides. These include *B. thuringiensis* Berliner, entomopathogenic fungi, entomopathogenic nematodes and granulosis viruses (Dreistadt, 2007). Fungal entomopathogens are important regulators of insect populations with considerable potential as mycopesticides. Fungal biocontrol agents have unique mode of infection. In contrast to the bacteria and viruses, they do not need to be ingested and can invade their host directly through the cuticle. That is why entomopathogenic fungi are capable of infecting non feeding stages like eggs (Ujian and Shahzad, 2007; Anand and

Tiwary, 2009) and pupae of insects (Nguyen et al., 2007; Anand et al., 2008). Moreover, fungal biological control agents have demonstrated efficacy against a wide range of insect pests including *Spodoptera* species (Purwar and Sachan, 2005; Lin et al., 2007; Amer et al., 2008). Application of entomopathogenic fungi against termites has a minimum negative impact on the environment. There have been a number of studies evaluating the efficacy of the hypocrealean Hyphomycete *Beauveria bassiana* (Bals.) Vuillemin, against subterranean termites and several other insects (Shahid et al., 2012). Similarly, Ascomycete, *Metarhizium anisopliae* Metsch Sorokin, present in the soil also acting as a causal agent for green muscardine of insects, is an important pathogen for the biological control of pests.

Different microbial antagonists like *Debaryomyces hansenii* Lodder & *Krejer-van Rij*, *Cryptococcus laurentii* Kufferath & Skinner, *B. subtilis* Ehrenberg Cohn, and *Trichoderma harzianum* Rifai, are being used. Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include, safety for beneficial organisms, reduction of residues in environment, and increased biodiversity in human managed ecosystems (Omkar, 2016).

The control of pests using natural enemies in protected cultivation relies mainly on the commercialized and imported natural enemies, i.e., *P. persimilis* in the 1970s and *E. formosa*, since the 1980s. However, the efficacy of these predators and parasites depends on various physical, chemical, biological, and environmental factors (Yano, 1993). For imported natural enemies, the arrival of the shipment at the right time along with maintaining the best quality represent two other critical factors that contribute to the success of natural enemies in the greenhouses. To overcome these limitations, indigenous

natural enemies can be used, since they are adapted to the domestic environment, and are highly active against their prey (Yano, 2003). Two examples of locally collected and mass-produced natural enemies, in this study, are the entomopathogenic fungus *B. pseudobassiana* and the predatory mite *P. persimilis*.

1. The Entomopathogenic fungus Beauveria sp. as a biological control agent of T. absoluta, B. tabaci and M. persicae.

a. Classification

The kingdom Fungi contains a diverse range of taxa with an estimated 1.5 million species of which 700 species from 90 genera have been described as insect and plant pathogens (Roberts, 1981). Many genera of entomopathogenic fungi are classified in the fungal class Hyphomyceta the anamorph of the Ascomycetes phylum (Polovinko, 2010).

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

b. Morphology

Beauveria bassiana (Hypocreales: Cordycipitaceae) produces dry, powdery conidia in distinctive white conidi balls (Fig. 5). Each conidia ball is composed of a cluster of conidiogenous cells. The cells of *B. bassiana* are short and ovoid and terminate in a narrow apical extension called a rachis. The rachis elongates after each conidium is produced, resulting in a long zig-zag extension, and forming a white mold (Rehner et al., 2011). *B. pseudobassiana* is phenotypically like *B. bassiana*, except for conidial size. The surface mycelium of *B. pseudobassiana* is cottony in texture with white colony margin and interior. Conidia are 2-3x1.5-2.5 µm in size and primarily subglobose or broadly ellipsoid in shape. Its vegetative hyphae are septate, branched, and hyaline (Rehner et al., 2011).

Figure 5. The growth status of *B. bassiana* isolate on PDA medium or host insects. (a) Muscardine weevil infected by *B. bassiana* found in a rice field. (b) *B. bassiana* separated from the muscardine weevil in the laboratory. (c) Adult *N. lugens* (48 h) after infection and death by *B. bassiana*. (d) Adult *N. lugens* (48 h) after death (Wang et al., 2018).

c. Life cycle

Asexually produced conidia of *B. bassiana*, germinate and penetrate the exoskeleton under favorable environmental conditions. Conidia germinate by means of a germ tube that penetrates by mechanical and enzymatic degradation, which allows the growth into the hemocoel. Growth continues by the formation of mycelium and hyphae, which colonize the host organs and hemolymph and secret toxins and secondary metabolites like beauvericin, leading to physical rupture of internal organs by vegetative growth of the fungi killing the host. Then, the fungus emerges from the cadaver and completes its life cycle by sporulation on the outside of the cadaver, causing white muscardine disease (Bateman et al., 1993).

d. Host Range

B. bassiana shows little specificity toward host range and can attack about 7 insect orders with more than 700 species (Vega et al., 2008). Four types of insect pests (beetles, termites, spittlebugs and locusts) are presently being controlled by this fungus (Shahid et al., 2012). It attacks a wide range of both immature and adult insects.

Previous studies proved the efficacy of the local isolate of *Beauveria* in controlling cedar web-spinning sawfly, *C. tannourinensis*, immature stages (egg and larvae), that were infected by the fungus and mortality rates ranged between 85%-100% depending on conidial concentrations (Abdo et al., 2008). Another study carried out by Abou-Jawdah et.al., (2008) showed, under laboratory conditions, that the local *Beauveria* isolate can successfully control the pine processionary moth, *Thaumetopoea wilkinsoni* Tams. Moreover, Shalaby et al., (2013) showed that no egg hatching of *T. absoluta* occurred when treated with the four conidial concentrations: 10^7, 10^8, 10^9 and 10^{10} conidia mL^{-1}. While, in

another experiment Inanli et al., (2012) reported that only 41.67% and 66.67% of Tuta eggs did not hatch. And recently, Al-Khoury (2020), proved the efficiency of the local *Beauveria* strain in the control of *T. urticae*. Under laboratory conditiions, 10^9 blastospores ml^{-1} led to the mortality of *T. urticae* life stages by 52, 67.9 and 95.3% in treated eggs, motile juveniles and adults, respectively. And in greenhouse experiments, higher *T. urticae* mortalities were recorded among strawberry plants (*Fragaria* × *ananassa*) sprayed with blastospores of *B. bassiana*.

e. Mass production

Extensive research is being conducted for improving fungal mass production and also to evaluate the effect of substrates, additives, and other factors on the virulence, viability, and thermotolerance of fungal conidia (Kassa et al., 2008; Machado et al., 2010). Diverse methods have been applied through time for mass production of entomopathogenic fungi.

i. Granular formulation

Most mass production schemes have utilized vegetable materials as the medium, e.g., rice or wheat bran, cracked barley, etc. A millet-based fungal production system has been reported (Gouli et al., 2008). The millet provides nutrition to support fungal growth in the soil in the absence of an insect host. This type of formulation is comparatively simple. Like any grain product, millet grains are placed in a polyvinyl bag, which is soaked in water containing citric acid and boiled at 90 °C for 1 h, after which the mixture is autoclaved at 121 °C for 30 min. The bag is incubated at 25 °C with a 16:8 h (L/D) photoperiod for 3 weeks. The culture is then air dried until it reaches a moisture

content of less than 5%. This process produces a granule with concentration of 1.1×10^8 *B. bassiana* conidia per gram of grain and a germination rate of 98.2% at 20 °C after 24 h. On the other hand, large-scale production of conidia in Russia is being accomplished by growing the fungi in a fermenter to produce large amounts of mycelia, which are then placed in shallow pans to a depth of approximately 1.0 cm where, after several days, conidia are produced. Research has shown that the type of substrate on which the fungus is grown can affect the thermotolerance of the conidia produced. The amino acid composition of the medium is known to influence the storage and virulence of conidia.

ii. Wettable powders

Wettable powder formulations (WP) of entomopathogenic fungi are most commonly produced, which contain 50–80% technical powder, 15–45% filler, 1–10% dispersant, and 3–5% surfactant (Burges, 1998). These are mixed with water and applied to the foliage as a standard insecticidal spray with ultra-low volume or hydraulic applications. It can also be applied to the soil as a drench.This formulation is being developed using a wide array of compounds, each with unique properties that affect particular factors to enhance efficacy or conidia survival. Additives are added that help to protect from UV light, enhance the ability of the conidia to stick to the foliage (reduce washing off of conidia), or increase humidity around the conidia to promote germination under adverse environmental conditions. Photoactive dye, phloxine B (0.005 g/m), has also been suggested (Kim et al., 2010b) to protect from phytotoxicity. Moisture has significant impact on conidial viability during storage and is also an important factor that affects shelf life. Potential application of moisture absorbent, like calcium chloride, silica gel, magnesium

sulfate, white carbon, or sodium sulfate, has been suggested in 10% WP conidial powder formulations.

iii. Oil formulation

In most of the formulations, a variety of oils are added to improve the shelf life of fungal products and to increase their field efficacy in dry climate. The use of oil as carrier helps to wet the waxy hydrophobic or lipophilic surface of insects and most plant leaves. The simple addition of oil to conidia powder increases the survival and viability of conidia (Moore et al., 1995). Oils also facilitate conidia adhesion to the insect, stimulate germination, and assist in penetration by disrupting the waxy layer of the cuticle. Isoparaffinic hydrocarbon solvents, such as paraffin oil and mineral oil, are being used as carriers for oil-based formulations. Other oils such as vegetable oils, methyl oleate (wetting agent and emulsifier), corn, and cottonseed oil have also been suggested (Kim et al., 2011). The viability of conidia in corn oil was over 98% for up to 9 months of storage at 25 °C and 23% at 21 months of storage.

iv. Method of application

Effectiveness of fungi as biocontrol agent depends upon the method of application. Four main methods of fungal applications are dipping the plant or roots, spraying the foliage, treating the soil, and indirect transmission by vectors.

Dipping of plant parts or roots in the conidia in the late afternoon, suspension of entomopathogenic fungi is one of the possible applications. However, this method is not very common. Foliar spray of fungal conidia in the late afternoon, to the site where the target insect occurs is commonly recommended to make fungal sprays in the late afternoon

to maximize the higher relative humidity that occurs at that time, which facilitates fungal germination. In a formulation of liquid culture of *B. bassiana*, a thermotolerant enzyme chitinase was mixed in order to enhance the pathogenicity (Kim et al., 2010a and Kim et al., 2010) and polyoxyethylene 3 isotridecyl ether (TDE–3) can be added as spreading agent.

Soil treatment of fungal conidia is probably the most practicable application strategy. However, it is suitable only if the target insect has a susceptible soil phase. In soil, the moisture level is sufficient to promote conidial germination and mycelial growth allowing fungal inoculum to sustain over time. Conidia in the soil are also protected from damaging UV light. Soil also helps to moderate large fluctuations in ambient temperatures, which often favors fungal growth and virulence. However, the antagonistic effect of soil pests (fungi, bacteria, arthropods) cannot be ruled out.

Several commercial formulations of *B. bassiana* have been developed for crop pest management. A list of commercially available fungi, target pests, and producer companies are presented in Table 2.

Table 2. Some Commercial Formulations of Entomopathogenic Fungal bio-Pesticides (Omkar et al., 2016)

Fungi	**Target pest**	**Crop**	**Product and company**	**Formulation**
B. bassiana	Sucking insects	Cotton, glasshouse crops	Naturalis™, Tray Bioscience, USA	Liquid formulation
B. bassiana	Coffee berry borer	Coffee	Conidia, AgroEvo, Germany, Columbia	Suspendible granules
B. bassiana	Whiteflies Aphids Thrips	Field crops	Mycontrol-WP/Mycotech Corp, USA	Wettable powder
B. bassiana	Corn borer	Maize	Ostrinil/Natural plant protection/France	Microgranules of mycelium

2. The predatory mite P. persimilis as a biological control agent for T. urticae

a. Classification

Phytoseiulus persimilis Athias Henriot (Mesostigmata: Phytoseiidae), is one of the predatory mites commercially available for the control of the two-spotted spider mites (Ghazy et al., 2014). The Phytoseiidae family is divided into four categories according to their feeding habits (McMurtry & Croft, 1997). Type I mites are specialized predators of heavily webbing spider mites, mostly *Tetranychus* spp. Type II species feed on spider mites, feeding on other small mites as well as on pollen and even on plant exudates. Type III, are generalists that often prefer prey other than spider mites such as tarsonemids and thrips. Type IV phytoseiid mites consist of members of *Euseius* species, generalist predators that develop and reproduce best on pollen and can become entangled within spider mite webbing. The significance of this system is in its application to the selection of phytoseiids for specific biological control targets. The species most often used in

greenhouses are *Phytoseiulus persimilis* Athias Henriot (a type I species), *Neoseiulus californicus* (McGregor) (type II) and *A. swirskii* (type III).

b. Morphology

The adults of *P. persimilis* are 0.5 mm (1/50 inch) long, characterized by the bright reddish-orange color, long legs and pear-shaped bodies (Fig.6). The immature predators are a pale salmon color, and they hatch from oval-shaped eggs (0.3 mm).

Figure 6. Female *P. persimilis* and its nymphal stage (Koppert®).

c. Life cycle

This predatory mite completes a generation within one week, during the summer season (Gerson et al., 2007) (Fig. 6), the female lays an average of 60 eggs over the 35-day lifetime, and the eggs hatch within 3-4 days depending on climatic conditions. Each predator consumes between 5-30 prey (eggs, nymphs, or adult stage) per day, except for the

newly hatched stage that does not feed. The *P. persimilis* can remain active year-round in greenhouses upon the availability of spider mites; it does not go through the diapause stage. The *Phytoseiulus* is known for its cannibalism phenomenon when it exhausts its food supply (Malais & Ravensberg, 2004). There are four times more *Phytoseiulus* females in the population than males (a sex ratio of 4:1) (Applied Bio-nomics, 1993).

d. Host range

Phytoseiulus persimilis (Acari: Phytoseiidae) is a specialist predatory mite used against spider mite species on vegetables and ornamentals (Ghazy et al., 2014). The introduction rates of *Phytoseiulus* differ depending on the infestation level of spider mites. It is better to start releasing the *Phytoseiulus* at the first sign of the spider mites. For example, it is recommended by Applied Bio-nomics© that the release ratio of predatory is 6 *Persimilis*/m2 and 2-4/m2 when the pest is first detected in greenhouse cucumbers and peppers, respectively.

e. Application of *P. persimilis*

Holding the necessary workshops, technical sessions, and training courses for growers as well as preparing appropriate brochures and agent-specific guidelines may enhance the applicability of biocontrol. A guideline on the release ratio of predatory mites points out the need of approximately 1 predator female (healthy) to 5 or 10 spider mites (all stages) for a rapid and effective biocontrol (Hoy, 2011). If our crop consists of 1000 plants with 30 leaves each, and each leaf averages 10 spider mites, we have approximately 300,000 spider mites and we need to release approximately 30,000 healthy predators with a

1:10 release ratio. In addition to that, it should be noted that development of the predator from one generation to the next is considerably shorter than that of the two-spotted spider mite. However, while this faster development is an important factor in its success as a predator, Griffiths (2000), noted that it is the initial ratio of pest to predator that determines the outcome of a control program. The basic data, according to (Scopes, 1985), for the time *P. persimilis* is expected to take to reach control over *T. urticae* is 16 days when the pest : predator ratio is 1:200, 13 days when it is 1:100, 11 days when it is 1:50, and 9 days when it is 1:50 (at 24°C). This clearly demonstrates that early application is more efficient and therefore more cost-effective (Kim and Sang, 2006).

F. Efficient use of Predators for Integrated Mite Management

The use of high-quality biocontrol agents for release is a fundamental step in the successful implementation of biocontrol programs (Fathipour et al., 2006). The main methods that are used in the evaluation of predators' effectiveness are life table parameters, foraging behavior, and multitrophic interactions. Studying the characteristics of predators helps to understand their influence on the population dynamics of prey and their influence on the structure of the mite communities in which they exist (Jervis and Kidd, 1996). Among life table parameters, the intrinsic rate of increase (*Rm*) is a key parameter in the prediction of population growth potential and has been widely used to evaluate efficiency of predators (Rahmani et al., 2009, Kianpour et al., 2011). In addition to the life table parameters, foraging behaviors and multiple interactions are useful tools for evaluation of mite predators (Farazmand et al., 2015, Farazmand et al., 2014, Maleknia et al., 2015, Maleknia et al., 2012) (Fig. 7).

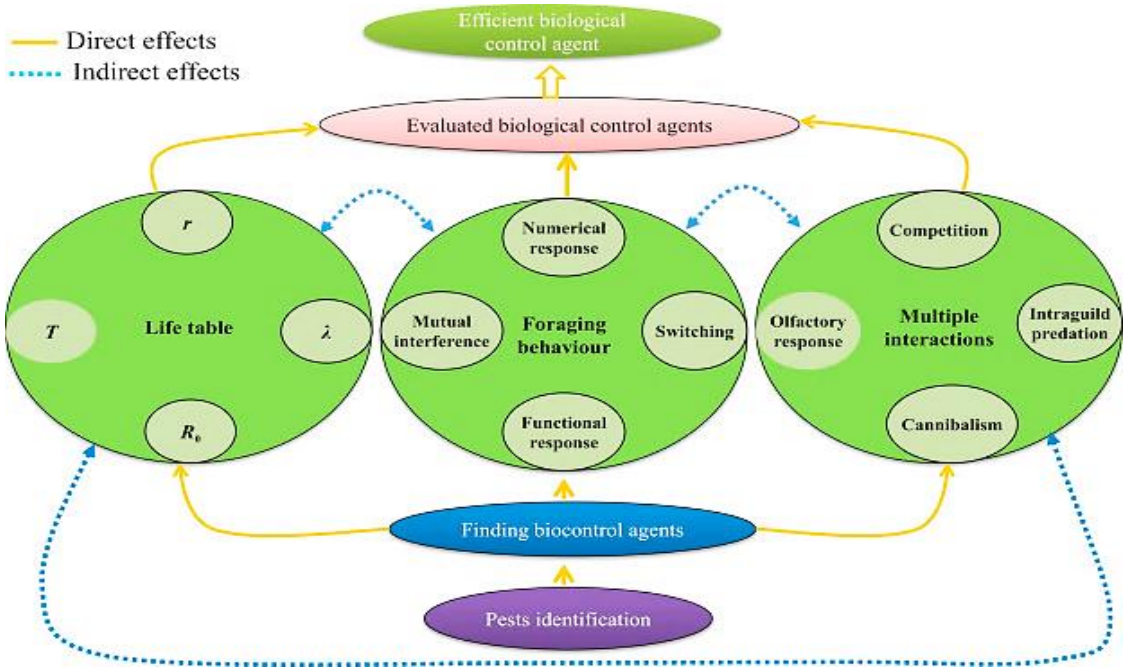

Figure 7. Important factors for evaluation of biological control agents (Omkar et al., 2016).

1. Life Table Parameters

Life table parameters shown in Table 3, influence population growth rates of a mite in the current and next generations. All life table parameters indicate the reproduction and population growth potential of mite predators, *r* is more important because other parameters are closely related to this parameter and it indicates the appropriate potential of the mite predator to increase its population; therefore, a higher intrinsic rate of increase shows higher potential of the predator for increase in its population. The intrinsic rate of increase can be affected by different factors, such as sublethal effects of acaricides (Hamedi et al., 2010, Hamedi et al., 2011), prey species (Escudero & Ferragut, 2005), host plant type (Khanamani et al., 2013; Khanamani et al., 2014), and temperature (Taghizadeh et al.,

2008; Ganjisaffar et al., 2011) (Table 3). It seems that an efficient predator should have a higher intrinsic rate of increase than its prey, although other traits, like predation capacity, are determinant in assessing the effectiveness of predators.

Table 3. The life table parameters of *T. urticae* and *P. persimilis* (Hoque, 2008).

Life table parameters	*Tetranychus urticae*			*Phytoseiulus persimilis*		
	Summer	Autumn	Winter	Summer	Autumn	Winter
Gross Reproductive Rate (GRR=$\sum m_x$) eggs/female/generation	52.50	65.51	42.38	24.0	31.4	21.3
Net Reproductive Rate ($R_0=\sum l_x m_x$) females/ female/generation	8.916	15.862	4.839	6.546	10.573	8.460
Capacity of increase ($r_c=[\log_e R_0]/T_c$) Female offspring/ female/day	0.1676	0.1735	0.0544	0.1747	0.1715	0.0960
Intrinsic rate of increase (r_m) eggs/female/day	0.1761	0.1873	0.0564	0.1823	0.1806	0.1025
Cohort Generation Time ($T_c=\sum x l_x m_x/\sum l_x m_x$) days	13.057	15.934	28.972	10.754	13.747	22.252
Generation Time ($T=[\log_e R_0]/r_m$) days	12.42	14.76	27.96	10.31	13.06	20.83
Finite Capacity for increase ($\lambda=\text{antilog}_e\ r_m$) Female offspring/ female/day	1.193	1.206	1.058	1.200	1.198	1.108
Doubling Time ($DT=[\log_e 2]/r_m$) days	3.936	3.701	12.290	3.802	3.838	6.762

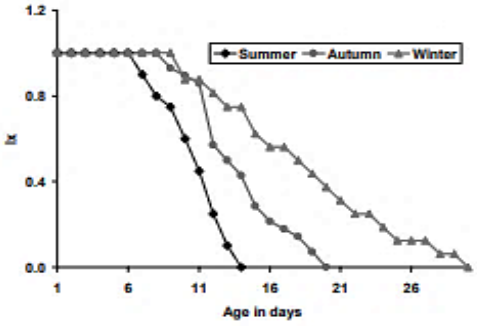

Fig. 1. Age-specific survival (*lx*) curves for *Tetranychus urticae* at three different seasons.

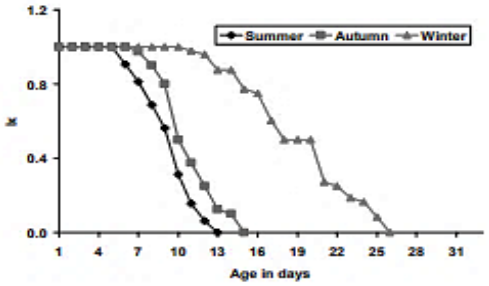

Fig. 2. Age-specific survival (*lx*) curves for *Phytoseiulus persimilis* at three different seasons.

2. Foraging Behavior

A basic understanding of the foraging behavior of predators and their prey aids predictions about predator function. Foraging behavior of predators, like functional

response, numerical response, mutual interference, and switching are usually affected by a number of factors, such as temperature, host plant, prey stage, experimental condition, and pesticides (Seiedy et al., 2012).

a. Functional Response

Functional response is the number of prey successfully attacked per predator as a function of prey density (Solomon, 1949). It describes the way a predator responds to the changing density of its prey. Holling (1959) considered three types of functional response. In type I there is a linear relation between prey density and the maximum number of prey killed, while in type II the proportion of prey consumed declines monotonically with prey density. Type III is described by a sigmoid relation in which the proportion of the prey consumed is positively density-dependent over some regions of prey density. A functional response models help to evaluate two vital parameters: handling time (i.e., the time needed to attack, consume, and digest the prey), and attack rate or searching efficiency. Predators with higher searching efficiency (a) and lower handling time (T_h) are better agents. Predators exhibiting the type III response are efficient biocontrol agents; nevertheless, many of the predators that have been successfully released as biocontrol agents have shown the type II functional response on their prey (Xiao and Fadamiro, 2010). *P. persimilis* is an important example of type II functional response, according to Seiedy et al., 2012. Functional response parameters of some mite predators are given in Table 4.

Table 4. The Functional response parameters of *P. persimilis* derived from selected literature (Omkar et al., 2016)

Species	Prey	Prey stages	*T* (°C)	*A**	T_{h}**	Reference
P. persimilis	*T. urticae*	Egg	25	0.130 h−1	0.49 h	Karimi et al. (2014)
P. persimilis	*T. urticae*	Egg	23	0.120 h−1	2.51 h	Maleknia et al. (2015)
P. persimilis	*T. urticae*	Larva	23	0.990 h−1	3.07 h	Maleknia et al. (2015)
P. persimilis	*T. urticae*	Larva	25	0.114 h−1	3.15 h	Seiedy et al. (2012)

*searching efficiency (a) , **handling time (T_h)

b. Numerical Response

The numerical response of a predator is a progressive change in the number of its progeny in relation to prey density (Solomon, 1949). It may be considered as a strategy of female predators to augment their offspring at different prey densities (Cédola et al., 2001). The efficiency of conversion of ingested food (ECI) reveals the relationship between conversion of prey biomass and prey density in which the ECI is more at low prey density and subsequently decrease at higher prey densities. This probably indicates that female predators at low prey density probably invest most of their energy in egg production and, in the process, invest less in maintenance and metabolic activities (Carrillo & Peña, 2012).

c. Mutual Interference

Aggregation by predators to prey patches is an integral component of models of prey–predator population dynamics. Hassell and Varley (1969) noted an inverse relationship between the predator density and searching efficiency. Inverse density dependence in searching efficiency is known as mutual interference. At increased predator density, individual predators will waste an increasing proportion of their searching time to encounter other conspecifics rather than handling prey. Reduced predation, running away, and hiding are some outcomes of interrupting a predator during search or capture of prey. Mutual interference occurs commonly in the laboratory (Pakyari and Fathipour, 2009; Farazmand et al., 2012, Farazmand et al., 2013), but it has rarely been reported in field studies. Understanding this mutual interference is necessary to predict the success of biocontrol programs, as it assists with mass-rearing efforts and can facilitate the explanation of observed outcomes in the field.

d. Switching

In predator–prey systems, switching plays an important role to increase the persistence of predator–prey systems in the long term. Switching occurs when predators change to alternative prey as the density of preferred prey starts decreasing (Aggelis et al., 2005). Several factors can lead to a type III functional response; one of the most important of these factors is the presence of alternative prey which can lead to a type III response through switching behavior (Buckel & Stoner, 2000).

e. Predation Capacity

To accurately evaluate the effect of predation in a predator-prey system, we need not only to assess the growth potential of the predator, but also its predation potential (Chi et al., 2011). The finite predation rate describes the predation potential of a predator population by combining its growth rate, age/stage consumption rate, and stable age/stage structure.

3. Multiple Interactions

Trophic interactions among primary producers (plants) and consumers (herbivorous and carnivorous mites) in the food web are the main fitness indicators of energy and nutrient cycle patterns through ecosystems. Plant quality can affect the fitness of herbivorous mites directly, as their food source, and indirectly, as foraging cues for their predators. The bottom-up effect of host plants can be extended to the third (first carnivores) and even fourth (second carnivores) trophic levels. Knowledge on bottom-up force of plants and top-down forces of predators is important for planning an IPM program, especially through decision making.

In complex food web systems (Fig. 8), predators can potentially interact negatively through competition, interference, and intraguild predation. All these interactions can affect directly or indirectly the success of biocontrol programs.

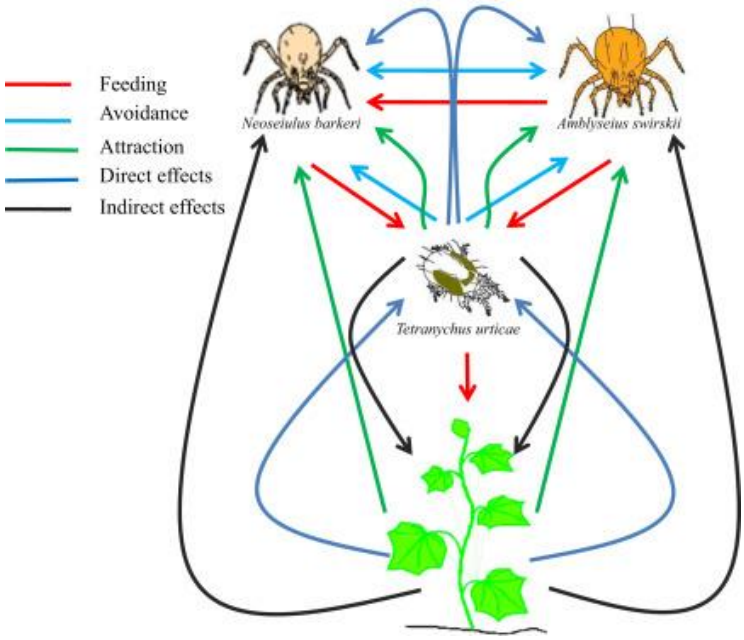

Figure 8. Tritrophic interactions in cucumber greenhouses (Omkar et al., 2016).

Intraguild predation (IGP) is a widespread phenomenon among arthropod food webs where more than one species feed on the same prey and therefore competitors feed on each other (Rosenheim et al., 1995). IGP occurs among wide varieties of natural enemies, such as phytoseiids and phytoseiids, and phytoseiids and thripids and can be affected by several factors, such as environmental conditions, host plant characteristics, mobility of prey, vulnerability of prey, feeding specificity, and presence of extraguild (EG) prey (Farazmand et al., 2015).

Cannibalism, the consumption of conspecific individuals, is a common phenomenon that occurs in insects. In the case of prey scarcity, feeding on conspecifics can be a choice for some species as alternative food source in order to survive and eventually reproduce.

4. The future of biological control of mite pests

Agricultural crops produced in a specific area are often exported to different parts of the world; therefore, growers are obliged to consider the approved international standards regarding the production of safe and residue-free crops or residues below the MRLs. Based on the high demand for safe food, a future high demand for biocontrol agents, especially mite predators, is predicted (Fig. 9). Therefore, more research and activities on different aspects of biocontrol as an ecofriendly practice are expected.

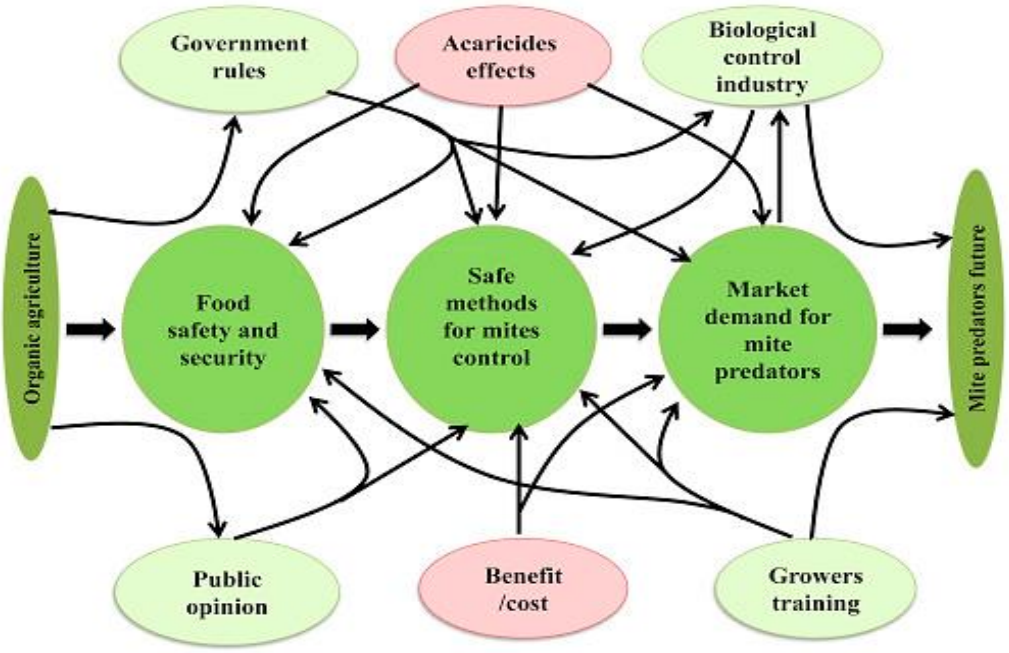

Figure 9. Factors affecting the future of mite predators (Omkar et al., 2016).

Commercial biocontrol is an industry where efficient natural enemies are mass-reared in biofactories for release in large numbers. Pilkington et al. (2010) expressed that in

greenhouse systems, *Encarsia formosa* (Gahan) accounts for 25% use in greenhouse, followed by the predatory phytoseiids *P. persimilis* and *N. cucumeris* (12%). This division of agents available to the greenhouse industry will, however, have changed significantly in recent years with the success of another phytoseiid, *A. swirskii* in Europe and North America.

The benefit to cost ratio is an essential tool for appropriate control strategies. This ratio for chemical control will be higher than for biocontrol if we consider the indirect costs of chemical control, like environmental pollution, human health problems, harmful side effects, and risk of pest resistance. Biocontrol is safe, sustains food security, improves food quality, reduces pesticide use, is safe to human health, controls invasive alien species, protects biodiversity, and maintains ecosystem services (Cock et al., 2010).

CHAPTER III

MATERIALS AND METHODS

A. Mass production and evaluation of the efficacy of *B. psuedobassiana* in the management of greenhouse pests

1. *Preparation of fungal suspension*

The local strain of *B. pseudobassiana* was used in this study. It was cultured on 9 cm diameter Petri dishes containing Potato Dextrose Agar (HiMedia®) at 25±2℃ and a photoperiod of 16 hours light and 8 hours dark. Conidia were harvested from two weeks old cultures by adding 10 mL of sterile distilled water and 0.01% Tween-20 (Sigma®). The conidia were scraped from the plate by a glass rod, and then the conidial suspension was filtered through three layers of cheesecloth. The resulting conidia suspension was used as a stock solution, and conidia concentration was calculated from conidia counts using the Neubauer hemocytometer. The appropriate dilutions were conducted to obtain the desired conidia concentrations: 10^5, 10^6 and 10^7 conidia mL^{-1}.

2. *Mass production trials of* B. pseudobassiana

Different substrates and water levels were used to determine their effect on conidis production. Rice, oat, un-hulled wheat, and coarse burghul were tested to determine the best substrate for conidia production (Fig. 10). For that reason, 600 g of each substrate were added in autoclavable plastic bags (30 x 60 cm). Distilled water was added to the substrates using 2 different water ratios, grain: water ratio (W/V), 1:1 and 1:0.5. The plastic bags were then shaken

well for the homogenous absorption of water, sealed using Food Saver Vacuum Sealer® and autoclaved at 121°C and a pressure of 15 bars for 16 minutes. When the temperature of the bags cooled down, each bag was inoculated by a 20 mL of 10^7 conidia mL^{-1} conidia suspension and incubated on their sides for 2 weeks (Fig. 11). Three replicates per treatment were used in each trial.

For conidia count, 1 g of each substrate was diluted in 100 mL of distilled water and the suspension was filtered through 3 layers of cheesecloth to remove impurities. Hemocytomteter was used to count the conidia using the following formula: average conidia count in the 5 squares (borders and one in the middle) x 250,000. The initial conidia concentration/g of culture medium was calculated by multiplying with the dilution factor.

Figure 10. Grains used for mass production trials, from left to right: coarse burghul, oats, un-hulled wheat, and rice.

Figure 11. Mass produced *Beauveria* bags: (a) one week old (b) two weeks old bags.

3. *Effect of adjuvants and additives on the conidia germination of* **Beauveria**

In order to determine the effect of additives and adjuvants on the germination percentage of *Beauveria* conidia, 2 experiments were conducted. First, the conidia suspension was prepared as discussed in section 1, and the concentration diluted to 10^5 conidia mL^{-1}. In the first experiment, mannitol was added to the conidial suspension in two different concentrations: 10g L^{-1}, 15g L^{-1} as an additive compared to the control treatment. In the second experiment the conidia suspension was amended with the following adjuvants: 1% corn oil, 0.25% vegetable oil (Stroller's natural oil ®, according to manufacturer recommendation), 0.25% LUQSA (nonionic surfactant, according to manufacturer recommendation), in addition to the control (distilled water). Each treatment was replicated 3 times and, in each replicate, 10µls of conidia suspension

was inoculated into PDA Petri-dishes and incubated at 25 ± 2 ℃ for 18 hrs at 16:8 photoperiod. Lactophenol blue was then added to the PDA medium and the conidia germination was recorded under a light microscope (40x). A conidium was considered germinated if the germ tube length was over 2x the conidium length.

4. *Evaluation of the efficacy of* B. pseudobassiana *against aphids and whiteflies, under greenhouse conditions*

a. Aphid rearing

Aphids, *Myzus persicae* Sulzer, were maintained on potted cucumber plants in insect-proof cages in the glasshouse at the American University of Beirut (AUB), under controlled climatic conditions. Data logger (Ebro®) was placed in the greenhouse to record daily temperature and RH, at an interval of 15 minutes. The rearing system of aphid was on cucumber plants of the "Alpha variety" at 25±2 °C, 60±5% relative humidity with a photoperiod of 16h light/8h dark in insect-proof cages. Adults were kept for 3 days for oviposition, and then the plants were removed into new cages for the development of adults. These adults were used to produce 2nd generation of same-age aphids. Adults of this generation were used in bioassays.

b. Whitefly rearing

Whiteflies, *Bemisia tabaci* Gennadius, were maintained on potted cucumber plants in insect-proof cages in the glasshouse at AUB, under controlled climatic conditions of 25±2 °C, 60±5% relative humidity with a photoperiod of 16h light/8h dark. Adults were kept for 3 days in the insect-proof cages for oviposition, and then the plants were removed into new cages for the emergence of adults. These adults were used to produce the 2nd generation of same-age whiteflies. Adults of this generation were used in the bioassays.

c. Preparation of large volume of fungal conidia suspension

Beauveria cultures (in Burghul, 1 kg/bag) were used in this experiment to produce a large volume of *Beauveria* conidia suspension. One-kilogram bag of Burghul is soaked in 1.5 L of water for 30 min, and then the solution was filtered through 6 layers of cheesecloth to remove all the impurities. On average, one liter of $1x10^9$ stock solution is left after the filtration, and the appropriate dilution was conducted to obtain the desired conidia concentration: $5x10^8$ conidia mL^{-1}. After that, the suspension was amended with 1% corn oil and 0.01% Tween- 20.

d. Bioassay: Small scale greenhouse experiment

To determine the efficacy of *Beauveria* conidia suspension on aphids and whiteflies, a small-scale greenhouse experiment was conducted. One-month-old cucumber plants of the "Alpha" variety were used, twenty aphid adults, and 20 whiteflies were released per cucumber plant and left for 3 days. During this phase, the cucumbers were covered with white agryl cover (floating row cover) (Fig. 12). The aphids were counted after 3 days in the form of aphid colonies, each colony consisted of about 13 aphids, roughly 4 adults and 9 instars. The eggs of whiteflies are difficult to count; therefore, we counted the 2^{nd} nymphal stage, 7-10 days after whitefly release (first appearance depending on climatic conditions). As for the results of aphids and whiteflies, the number of insects was recorded on 3 leaves per cucumber plant (lower, middle, and upper level) in both the control and *Beauveria* treatments. Three sprays took place on 5-day intervals and the treatments applied were $5x10^8$ conidia mL^{-1} + 1% corn oil + 0.01% Tween-20; while, the control treatment consisted of 1% corn oil + 0.01% Tween-20. A total of six replicates per treatment, each consisting of one cucumber plant, were used in this experiment. The climatic conditions where average temperature of 19℃ and 78% RH.

Figure 12. Cucumber plants covered with white agryl during insect release

e. Statistical analyses

Multivariate analysis was conducted, using IBM SPSS statistics 25 to assess the efficacy of *Beauveria* treatment compared to the control.

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

a. *T. absoluta* rearing

Tuta absoluta larvae were collected from tomato greenhouses located in Kfardonin region, South of Lebanon. The insects were released and reared on potted tomato plants placed inside insect-proof cages (55 cm x 55 cm x 55 cm), in a glasshouse, at AUB. Dead tomato plants were replaced by new, healthy ones to maintain *T. absoluta* population. *T. absoluta* adults

(Fig. 13) were collected using a cordless vacuum cleaner (Bosch®). Throughout the time of insect rearing, the temperature ranged between 18-35°C, and the RH ranged between 40-90%.

Figure 13.Collected *T. absoluta* adults from the rearing system

b. Bioassay

Treatments

In the following experiments, two treatments were applied, the *Beauveria* treatment which consisted of $5x10^8$ conidia mL^{-1} (prepared as described in section 4.c) + 1% corn oil + 0.01% Tween-20 and the control treatment was 1% corn oil + 0.01% Tween-20.

Cage method

In insect-proof cages, one-month-old tomato plants of the "AUB-Line 9" variety were used for this experiment. The treatments studied consisted of 3 replicates, each consisting of 1 tomato plant. Five *T. absoluta* adults were released per replicate and left for 4 days to lay their eggs, then the number of eggs per plant was recorded (day 1). Depending on the climatic

conditions and the hatching period of the eggs, the first spray took place after egg counting directly (day 1), and the second spray was applied after 8 days. The eggs then hatched on day 12. The climatic conditions where average temperature 20 ºC and 70% RH.

Greenhouse method

A netted greenhouse, planted with tomato plants of the "AUB- 9" variety, was used for this experiment during December 2018 (Fig. 14). This trial included only two treatments: *Beauveria* and control treatments that were replicated 3 times; each replicate consists of 1 tomato plant (3 months old). Thirty *T. absoluta* adults were released per plant and were left for 4 days to lay their eggs. The first spray took place after egg counting directly (day 1), a second spray was applied after 13 days and the eggs hatched on day 26 days. The climatic conditions where average temperature 14 ºC and 75% RH.

Figure 14. Tomato plants covered with white Agril during *T. absoluta* release

For determination of the efficacy of *Beauveria* treatment in both experiments (Cage and greenhouse), results were recorded as non-hatched eggs and hatched eggs. The larvae from the hatched eggs were monitored for mortality rate and recorded as either healthy/living when leaf mines were more than 5 mm long or dead when the mines were less than 5 mm long. Corrected mortality (CM) used when the egg mortality in the control treatment is between 5 and 20%. It was calculated using the Abbott formula (Abbott 1925).

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

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

c. Statistical analyses

A total number of 347 *T. absoluta* eggs were used to conduct both experiments. One-way ANOVA, using IBM SPSS statistics 25, test was performed on experimental data to assess the control efficacy of the treatments applied against *T. absoluta* eggs.

B. Mass production and evaluation of the efficacy of *P. persimilis* for the management of *T. urticae*

1. Survey and collection of Phytoseiid mites

Surveying for Phytoseiid mites was organized in two seasons of the year 2018-2019, the first was during late winter (low temperature and high humidity) and the second was during spring season (mild temperature and high humidity). Fifty symptomatic leaves infested with *T. urticae* mites were collected at each sampling site. Leaves from each site were placed inside zip plastic bags, marked and transported to the laboratory inside a portable cooling box. In the

laboratory, a stereomicroscope was used to inspect the leaves. The phytoseiid mites found on the leaves are then transferred and reared in insect proof cages, each containing cucumber plants infested with *T. urticae* as a prey.

2. Morphological identification

Twenty phytoseiid adult mites from each location were transferred to 1.5 mL Eppendorf tube containing 75 % ethanol, using a wet painting brush. The samples were then sealed with parafilm and sent to Dr. Ziad Barbar at University of Homs, Syria and to Dr. Frederic Beaulieu at Ottawa Research and Development Centre (ORDC), Canada for morphological identification.

3. Molecular identification

Molecular identification was done at the plant pathology lab at AUB- FAFS. The mites were preserved in 95% ethanol for DNA extraction. Total Nucleic acids extraction was carried out starting from one adult mite with a cetyl-trimethyl ammonium bromide (CTAB) method. Samples were placed in 1.5 mL microcentrifuge tubes, flash frozen in liquid nitrogen and ground with a microcentrifuge pestle and drill. An aliquot of 800 µl of 2% CTAB buffer (Appendix I) supplemented with 16 µl of β-mercaptoethanol was added onto each ground sample. These samples were incubated in a water bath at 60ºC for 20 min with mixing byvortexing every 5 min. Subsequently, 600 µl of isoamyl alcohol-chloroform (1:24 v/v) were added to the mixture, vigorously vortexed, and centrifuged at 10,000 rpm for 5 min in a Centrifuge 5804 R (Eppendorf). The supernatant was transferred to a clean microcentrifuge tube and 600 µl of ice-cold isopropanol were added. This mixture was kept at -20ºC for 30-60 min and later centrifuged at 14,000 rpm for 8 min. The supernatant was discarded, and the pellet was washed with 70% ethanol and centrifuged at 14,000 rpm for 5 min. The supernatant was discarded, and the ethanol

was evaporated using a vacuum concentrator. The pellet was dissolved in 50 µl autoclaved distilled deionized water and stored at -20ºC.

4. PCR, cloning and sequencing

The ITS region was targeted using the primer pair 5'AGAGGAAGTAAAAGTCGTAACAAG 3' for the 3' end of 18 rDNA and the 3'ATATGCTTAAATTCAGGGGG 5' 5' end of the 28S (Navajas et al., 1999). The PCR reactions were performed in 20 μL reaction volumes containing 20ng/μL of sample DNA, 4 μL of 5x FIREPol® Master Mix Ready to Load (Solis BioDyne,Estonia), 10 picomol of each primer and 12 μL of distilled deionized water. Amplifications of the ITS gene were performed in C1000 Touch^{TM} thermocycler (Bio-Rad, USA) at 92℃ for 2 min followed by 35 cycles of 92℃ for 15 sec, 50℃ for 45 sec, and 72℃ for 1 min, and a final extension step at 72℃ for 7 min. The PCR products were run on 1% agarose gel and were visualized under UV following staining with ethidium bromide. Following agarose gel electrophoresis, the PCR amplified products were purified using the QIAquick Gel Extraction Kit (Qiagen, Germany) according to manufacturer's instructions. The PCR products were cloned in pGEM-T easy vector (Promega, USA) and sequenced. Sequencing was done at the "Unité de Génétique Médicale", University of Saint Joseph, using an automated DNA sequencer. The resulting nucleotide sequences were compared to the published sequences of *P. persimilis* at NCBI, using the Blastn program.

5. Mass production of P. persimilis

The production cycle of *Phytoseiulus* requires 3 separate glasshouses, the first greenhouse is dedicated to produce seedlings, the second one is used for the rearing of spider

mites and the third one for the mass production of *Phytoseiulus*. It needs a period of 6-7 weeks to complete a cycle. For continuous production, overlapped cycles can be established.

a. Seedlings glasshouse

A glasshouse compartment maintained under controlled climatic conditions (25± 4°C and 70-90% RH) was used for production of seedlings. Rearing insects took place on three main crops beans, cucumbers and eggplants, the latter in a separate greenhouse.

During the fall and winter seasons, beans of the "Jinane" variety were grown for the production system. While during spring and summer seasons, cucumber plants of the "Majed" variety were preferably used. Cucumber can withstand the hot climatic conditions better than bean. In parallel, eggplants of the "Nigra" variety were specifically cultivated throughout the year for the continuous rearing of spider mites. These crop varieties were chosen for their rapid growth, ability to withstand high levels of spider mite infestation and extreme climatic conditions. Seeds were either coated with metalaxyl or thiram for early disease control. Seeds were sown in the planting mix (Sab substrate®, Germany).

The peat-based planting mix Plantafior® (USA) was then used for transplanting in small pots. Sawdust is added to the mix to increase aeration and to reduce the cost of the growing media. Cucumber and eggplant seedlings were transferred as 1 seedling/ pot. However, the beans were transferred as 2 seedlings/ pot. At this stage, irrigation was manually done by an irrigation hose. After 4 weeks, the seedlings (6 true leaves/plant) were ready for transfer to the second glasshouse.

b. *T. urticae* stock culture

A strain of *T. urticae* collected from Jiyeh region was selected as a starting culture to produce spider mites stock culture as a source of feed for the production of *P. persimilis*. Every year the mite stock culture was renewed, by collecting spider mites from the field.

c. Spider mite production greenhouse

A separate greenhouse (15 $m^{2,}$ containing about 200 pots) was dedicated to produce spider mites. For continuous rearing, spider mites-infested leaves from the older series of plants were removed and deposited near the stem base of the new series of plants. The target number of spider mites for re-culturing is about 75-125 eggs + mobile stages for the infestation of 5 plants in the new series under optimal climatic conditions (18 hours light, 28℃ and 40% RH). This procedure is repeated on a weekly basis. Once the inoculum leaves have dried out and the mites have moved on to the new plants (3-4 days), the dried leaves were removed and discarded.

d. *Phytoseiulus* stock culture

The *Phytoseiulus* strain was collected from bean plants grown in a conventional greenhouse in Jiyeh region- South Lebanon. The local strain is expected to be adapted to normal conditions of the Lebanese coastal region. In addition, the farmer reported that he was following a biweekly spray program using pesticide mixtures; accordingly, this strain may have been subjected to different pesticides and acaricide sprays.

d. *Phytoseiulus* production greenhouses

A single span greenhouse equipped with 6 isolated benches of about 3.2m^2 each (Fig. 15) is used for the mass production of *Phytoseiulus*. A total of 24 cucumber plants grown in individual pots were placed on each bench (4 rows of 6 plants each). Cucumber plants,6-8 weeks old become normally ready for infestation with spider mites. 60 spider mites / plant were released and left to reproduce for one week. Then, the counting of the spider mites took place and the release of *Phytoseiulus* was done according to the ratio 40: 1, *T. urticae: P. persimilis*. Counting of *Phytoseiulus* takes place at weekly intervals. The cycle should continue in an overlapped program for continuous predator production.

The optimum lighting and humidity for *Phytoseiulus* is: 16 hours light, 25℃, 70-80%RH. Healthy female *Phytoseiulus* will lay an average of 2 eggs per day. One week later, the series is examined for purity (absence of different pests) and to check the balance of *P. persimilis*: *T. urticae* ratio. This can be done by taking a sample of leaves and examining them under a microscope. Ideally there should be low numbers (20-30 per leaf) of *Phytoseiulus* and good numbers of spider mite eggs and mobiles (most of the leaf area will be covered with mites but the leaves should still be green). This will vary throughout the series and it may be necessary to add more spider mite to some areas. Adding a nutrient boost to the plants will also affect the balance as it will cause increased production of new leaves and lower the overall density of spider mites.

Figure 15 *P. persimilis* production greenhouse

e. Harvesting

On the 2nd or the 3rd week after predator inoculation, the *Phytoseiulus* should have eaten most of the prey. Once most of the spider mites have been eaten the female *Phytoseiulus* begin to migrate to the tops of the plants and some areas of the new leaves will appear to have no mite damage. At this point and before they leave the plants specialized *Phytoseiulus* collectors are placed over the tops of the bean/ cucumber plants in these areas (Fig. 16). These bottles are checked at least twice a day depending on activity and temperature and the predators, removed, counted, and placed in cold storage. In addition, leaves can also be harvested for and distributed to farmers as *Phytoseiulus* leaf product (Fig. 17). The mites on leaves are counted under a microscope (40x magnification) and placed in plastic trays. This is done by scanning the leaves and selecting the ones with the most homogenous distribution of predators.

Figure 16. Harvesting Phytoseiulus using the bottle technique

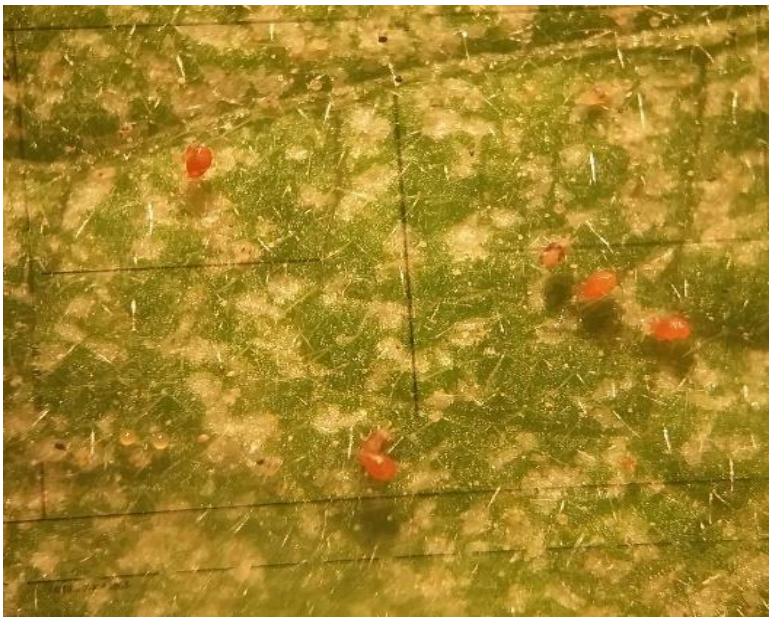

Figure 17.*Phytoseiulus* on leaf product and counting them under light microscope (40x).

f. Counting method/quality control method

The predatory adults that have moved into a collection bottle are counted by estimating the number of *Phytoseiulus* inside the bottle. The adults clump together and the density and size of the clumps with practice can be used to estimate the numbers. Then 10cc of vermiculite is added per 1000 predators per bottle, the bottle was closed and gently swirled so as to "wash" the predators off the inside of the bottle. The collected predators and vermiculite carrier are then poured into a bottle, and small pieces of paper towel (1cm.sq./10,000 mite) soaked in water was added to container of *Phytoseiulus* to provide moisture.

g. Pest management in the *Phytoseiulus* production

All greenhouses openings should be covered with insect-proof screen. Plants may become contaminated with whiteflies, thrips, and aphids. Weekly monitoring must be used to check for contamination. Whiteflies may be controlled by using yellow sticky traps. Aphids

may be controlled by using pirimicarb and by removing infested plants or plant parts. Thrips and caterpillars may be controlled by fumigating with dichlorvos but cannot be used once *Phytoseiulus* is added. Spraying spinosad, against infestation of thrips is possible in the presence of *Phytoseiulus*, however it is slightly toxic (Appendix II, Table I) created by combining info from Koppert© and Biobest© groups). Most other insect plant pests are not a problem due to the short growth cycle and a thorough greenhouse bench clean up between crops.

To control root diseases the planting beds are drenched with propamocarb, upon transplanting. To prevent the spread from series to series, the pots are washed and disinfected between each series with soap solution and bleach solution.

6. Best rearing ratio of pest: predator for mass production

The best *T. urticae: P. persimilis* ratio **r**ecommended by Applied Bio-nomics© for the commercialization and delivery of mites to farmers is 1:3.

In order to achieve the objective of the best ratio of rearing mites, the following experiment was conducted. In a controlled greenhouse conditions (25±2℃, 70-80% RH). The experiment consisted of 4 different ratios of spider mite: *Phytoseiulus* (0, 20:1,40:1,60:1). Twelve bean plants of the Jinane variety, at the stage of 3-true leaves, were infested with 20, 50, 100, 150 spider mites/plant in the control, 20:1, 40:1, and 60:1 ratios, respectively. One-week post release, the number of spider mites was counted on 8 leaves (week 0), and the predatory mites were released according to the different ratios. The experiments consisted of 4 treatments, 20:1 ratio releasing 1 *Phytoseiulus* for every 20 spider mites, 40:1, 60: 1 and the control where there are only spider mites. Each treatment was replicated 3 times (3 plants per replicate). Monitoring continued at weekly intervals until reaching the ratio 1:3.

7. *Efficacy of the local* **P. persimilis strain** *for the management of spider mites in a small greenhouse within an IPM strategy.*

The objective of the following two experiments were to evaluate the efficacy of a local strain of *P. persimilis* against *T. urticae* and to determine its compatibility with a local isolate of *B. pseudobassiana* intended for aphid and whitefly control within an integrated pest management program under greenhouse conditions, the experiment was performed in a greenhouse cultivated with cucumber plants of Majed variety. The treatments were untreated control, 1% corn oil +0.01% Tween-20 and 10^8 conidia mL^{-1} + 1% corn oil + 0.01% Tween-20 suspension. Each treatment consisted of 3 fully mature plants and data were collected by reading 3 leaves per/ plant (upper fully expanded, middle, lower). Fifty spider mites were released per cucumber plant and kept for 1 week to reproduce. One-week post spider mites release (day 0), the number of spider mites was counted and the *Phytoseiulus* was released at a rate of 12 adults/ plant. At day 7, the first spray of treatments took place followed by a second spray at day 14. Then monitoring of mite population continued till day 21.

8. *Efficacy of the local* **P. persimilis strain** *for the management of spider mites in a small greenhouse within an IPM strategy in insect-proof cages*

In order to determine the efficacy of a local strain of *Phytoseiulus persimilis* against *T. urticae* and its compatibility with a local isolate of *B. pseudobassiana,* 15 cages of fine mesh were placed in controlled climatic conditions 25±2℃ and 70-80% RH. The six treatments were untreated control, 1% corn oil + 0.01% Tween-20 (adjuvant control), *Phytoseiulus*, *Phytoseiulus* + 1% corn oil+ 0.01% Tween-20, *Phytoseiulus* + 10^8 conidia mL^{-1} + 1% corn oil + 0.01% Tween-20, and 10^8 conidia mL^{-1} + 1% corn oil + 0.01% Tween-20 alone. Each treatment was replicated three times, and each replicate consisted of 3 cucumber plants. The plants were 5

weeks old, when 20 spider mites/ plant were released and kept for one week to establish. One week later (day 0), the number of spider mites was counted, followed by the release of *Phytoseiulus* at a rate of 12 predatory mite/ cage. Then, the sprays of *Beauveria* suspension and oil emulsion were done at day 7, followed by a second spray at day 14. Monitoring of mites and their predator continued till day 23 in all the treatments (Fig. 18)

Figure 18. Insect-proof cages used for the experiment under glass house conditions.

9. ***Large scale field trials in commercial greenhouses to evaluate the efficacy of the local strain of P. persimilis in the management of T. urticae within an integrated pest management system.***

a. Experimental site

In a farm located in Kfarmashoun – Jbeil district (Fig. 19), situated at 300 m above sea level, two greenhouses of 450m^2 (9x50m) were chosen for this experiment. The greenhouses

were equipped with 12 irrigation pipes; ten rows were cultivated with 1000 cucumber plants of "Serena" variety and 2 rows with 200 colored pepper plants of the "Tala" variety on the borders (Fig. 20). The planting density was 2 plants per m^2.

Figure 19. Kfarmashoun experimental site (yellow arrow)

Figure 20. Distribution of cucumber and pepper plants in Kfarmashoun greenhouses.

b. Pre-transplanting measures in IPM greenhouses

Both greenhouses were equipped with clear polyethylene covers and the vents were closed by insect-proof nets. Data loggers (Ebro®) were installed to record daily temperature and relative humidity, at an interval of 15 minutes. The soil was prepared by fumigating it with Allyl Isothiocyanate (Dominus®) before the beginning of the growing seasons of Fall-Spring 2019. In both greenhouses, pepper plants were left on the borders from the previous growing season (Fall 2019). However, the plants were pruned to eliminate or reduce the overwintering stages of insects. For the elimination of recently introduced or any remaining whiteflies, thrips and fungus gnats from the IPM greenhouse, yellow sticky cards (YSCs) and blue sticky cards (BSCs), provided by the Ministry of Agriculture extension services, were installed at a rate of 1 trap/16m^2. About a week before transplanting, trap plants, in the form of blossomed marigold pots, were placed in the IPM greenhouse at a rate of 1 flowering plant per 32 m^2. Each pot contained 1 yellow marigolds (Ferry-morse®) (Fig. 21), that was grown in a nursery and was not treated with any type of insecticide. One day before cucumber transplanting, marigolds were covered by a nylon bag and discarded to eliminate any insect that may have entered the greenhouse and was attracted to the trap plants. New flowering marigolds replaced the removed ones. Weeds, inside and around the greenhouses, were pulled by hands instead of applying herbicides and plant debris left from last season were removed and burned. Cucumber plants were transplanted on March 6, 2019.

Figure 21. Trap plant, flowering marigold

Figure 22. Insect scouting

c. Post-transplanting measures

i. Insect/mite scouting

Weekly insect/mite scouting was performed in both greenhouses (Fig. 22

Figure *22*). A total of 50 randomly selected cucumber plants and 30 pepper plants were monitored per greenhouse. From each selected plant, 3 leaves were randomly scouted; that is a total of 150 leaves of cucumber and 90 leaves of pepper per greenhouse. The number of *T. urticae* nymphs and adults, in addition to adults and instars of *B. tabaci* were recorded. The same scouting technique was used also for the monitoring of *P. presimilis* population. The *Phytoseiulus* was released on regular basis for the control of *T. urticae*; while, *Beauveria* conidial suspensions were sprayed against aphid population as a hot spot treatment on peppers as the peppers were found on the borders of the greenhouse. Throughout the growing period, highly infested marigolds and cucumber leaves/plants were removed from the IPM greenhouses. New flowering marigolds replaced the removed ones.

The farmer had to stop production in the control greenhouses on 21/6/2018. The data collected on temperature and relative humidity are provided in Appendix III.

ii. Control greenhouses

The farmer spraying program included at least 14 pesticide sprays during the growing season, each consisting of a mixture of minimum of 3 pesticide active ingredients, applied roughly at weekly intervals. Some applications contained up to 4 different pesticides in one spray. Main sprays were insecticides/acaricides targeting mites, thrips, and whiteflies, while the Fungicides were targeting mildews. Details of pesticides applied in the control greenhouse,

including dates of application, active ingredients, and trade names, are provided in Appendix II, Table II.

iii. IPM greenhouses

After transplanting of cucumber plants and before the beginning of our trials, the farmer had used 2 sprays of pesticides (Emamectin Benzoate, Acetamiprid and Fenbutatin oxide) as a foliar spray. At week 0 (March 27/2019), we started by releasing 10000 *Phytoseiulus*, as a preventive measure. Throughput the season, c hlorothalonil, classified as a safe fungicide for predators, was applied as a foliar spray for the control of mildews. Details of pesticides applied in the IPM greenhouse, including dates of application, active ingredients, and trade names, are provided in (Appendix II, Table III).

The average number of insects/leaf was calculated and presented. In addition to that, the intrinsic rate of increase was also calculated where: $r = \frac{N2-N1}{t2-t1}\frac{1}{N1}$

N_2 is the final population size, N_1 is the initial population size and *r* is the intrinsic rate of increase, t_2 is the time final and the t_1 is the time initial (Elzinga, 2014).

d. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics 25. Statistical analysis evaluated the efficiency of control of each beneficial organism to its corresponding pest, by comparing the population of each insect in the IPM greenhouse to its population in the control greenhouse, using two-way ANOVA.

CHAPTER IV

RESULTS AND DISSCUSSION

A. Mass production and evaluation of the efficacy of *B. psuedobassiana* in the management of greenhouse pests

1. Mass production trials of B. pseudobassiana

Four different grain substrates were tested for the mass production of *B. pseudobassiana* at two different ratios of grains to water (W/V). Table 5 shows that at 1:1 ratio, burghul and oats gave the highest concentration of conidia, $3x10^9$ and $2.33x10^9$ conidia g^{-1}, respectively, without a significant difference between the two substrates (P=0.06), after two weeks of fungal inoculation. However, these concentrations were significantly different from the conidia produced by burghul and oats at 1:0.5 ratio, and from wheat and rice at both ratios. In the 1:0.5 ratio, the concentration of conidia harvested from burghul was $1.1x10^9$ conidia g^{-1} and $1.06x10^9$ conidia g^{-1} on oats (P burghul=0.000, P oat=0.007). On the other hand, wheat produced $0.27x10^9$ and rice gave $0.93x10^9$ conidia/ g^{-1} at 1:1 ratio, while producing $0.62x10^9$ and $0.93x10^9$ conidia g^{-1} at 1:0.5, respectively. For wheat and rice, there was no significant difference in the concentration of conidia produced at the 1:1 and 1:0.5 ratio (P wheat= 1.000, P rice=1.000). In addition, there was no significant difference in the conidia yield between wheat, rice, burghul, and oats at 1:0.5 ratio (P =1.000).

This shows that, burghul and oats can be used for the mass production of *Beauveria* conidia, at 1:1 water to grain ratio. Burghul grains are less expensive compared to oats, and they

do not need as much labor work compared to oats. In addition, the texture of solid grain substrate was more favorable and practical for use when 1:0.5 ratio was applied. The grains maintained their solid shape and the growth of fungi was more homogenous over the substrate.

The conidia production in our experiments are of the same order of magnitude to those reported by Ibrahim et al., (2017), who reported, high conidial yields of around $4.0x10^9$ conidia g^{-1} for *B. bassiana* cultured on spoiled cereals, ad $2.0 - 4.0x10^9$ conidia g^{-1} were collected from potato and carrot peel substrates.

Table 5. Mean number of conidia g^{-1} produced by *B. pseudobassiana* on different solid grain substrates at grain water: ratio of 1:1 and 1:0.5.

Grain: Water ratio	**Grain**	**Concentration*10^9 conidia g^{-1} ± SE*10^9 ***
1:1	Rice	0.93 ± 0.66a
	Oat	2.33 ± 3b
	Wheat	0.27 ± 0.33a
	Burghul	3 ± 3b
1:0.5	Rice	0.93 ± 0.97a
	Oat	1.06 ± 1a
	Wheat	0.62 ± 1a
	Burghul	1.1 ± 1a

* Mean concentration ± standard error (SE); different letters indicate statistically significant differences according to Bonferroni's test ($P \leq 0.05$).

2. *Effect of adjuvants and additives on the conidia germination of Beauveria*

In attempts to boost the conidia germination of *B. pseudobassiana*, different additives and adjuvants were applied to the conidia solution. Addition of mannitol at two different concentrations (10 and 15 g/L) to *Beauveria* conidia suspensions gave a significantly higher percentage of conidia germination compared to the control treatment after 18 hours of incubation. In the control treatment only 65% of conidia germinated, while in the 10 and 15g/l of mannitol 83.66% conidia germinated in both concentrations. There was a significant difference in the germination percent between the control and added mannitol (P=0.002). While, there was no significant difference between the two added concentrations of mannitol (P=1.00) (Fig. 23). This shows that the added sugar improved the percent germination of *Beauveria* conidia.

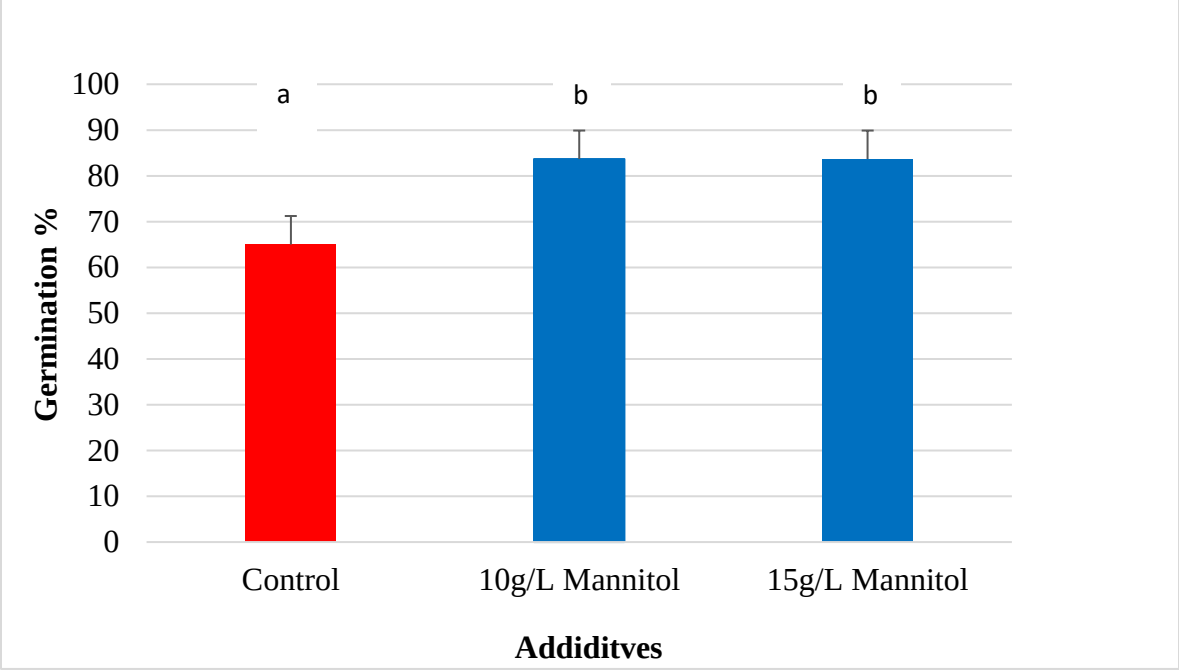

Figure 23. Average percent germination of *Beauveria* conidia with and without mannitol, after 18 hours of incubation. Different letters indicate statistically significant differences according to Bonferroni's test (P≤0.05).

When evaluating the impact of adjuvants on conidia germination, the highest germination percent observed after 18 hours of incubation, was 93% when 1% corn oil was added to the conidia suspension, which was significantly higher than all the other treatments: control (85%), 0.25%LUQSA (79%) and 0.25% Vegetable oil (76%) (Fig. 24). There was a significant difference in the germination percent of *Beauveria* conidia when different adjuvants were added (P=0.000).

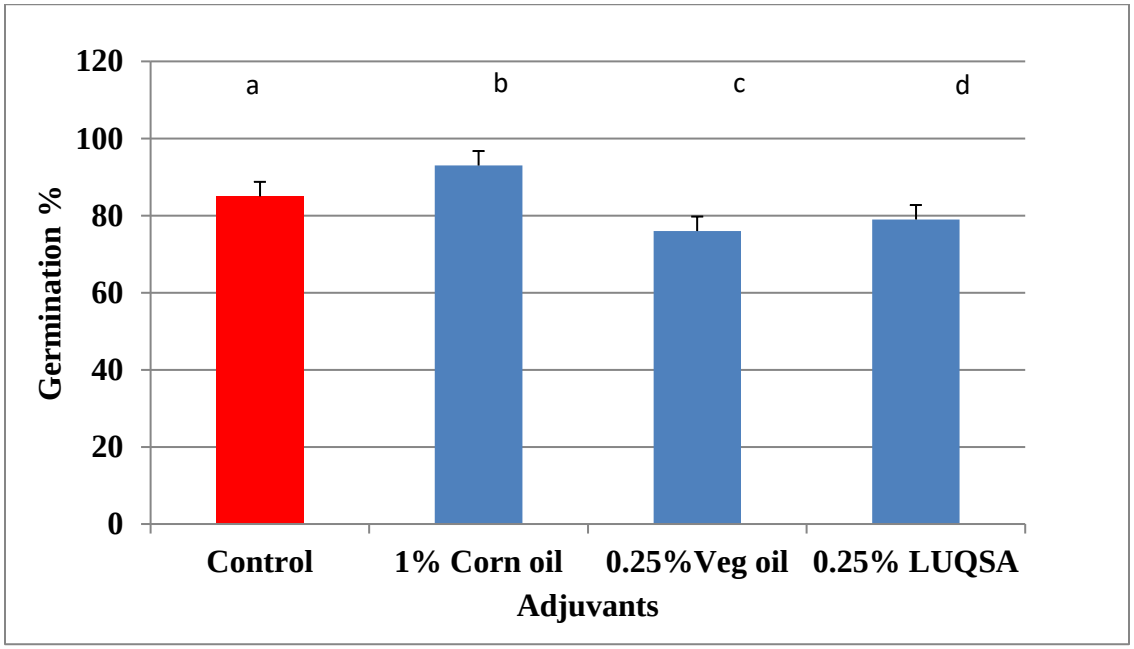

Figure 24. Average percent germination of *Beauveria* conidia with and without adjuvants, after 18 hours of incubation. Different letters indicate statistically significant differences according to Bonferroni's test (P≤0.05).

3. *Evaluation of the efficacy of B. pseudobassiana against aphids and whiteflies, under greenhouse conditions*

a. Aphid control

Three days after aphid release (day 0), there was 2.66 and 2.05 aphid colonies/ leaf in the control and *Beauveria* treatments, respectively. After the first spray (day 5), the aphid population

increased in both treatments and the second spray was applied. Then, at day 10, the population of aphids reached its highest peak of 16.27 aphid colonies/ leaf in the control, while it decreased in the *Beauveria* treatment to reach 2.77 aphid colonies/ leaf at day 10 (83% reduction as compared to the control). After the third spray, the aphid population decreased by half in both treatments to reach 8.61 and 1.27 aphid colonies/ leaf in the control and *Beauveria* respectively (85% reduction as compared to the control). In addition, signs of whitish mycelium on aphids was recorded in the *Beauveria* treatment (Fig. 25). This shows that, two sprays of *Beauveria* resulted in limiting the reproduction and spread of aphids, while the oil treatment required 3 sprays to slightly manage the aphid population (Fig. 26). There was a significant difference in the number of aphid colonies between the two treatments and in the days*treatment (P=0.000).

Figure 25. Aphids infected with *B. pseudobassiana,* showing whitish fungal growth and sporulation under greenhouse conditions, after 2- sprays with the fungus conidia

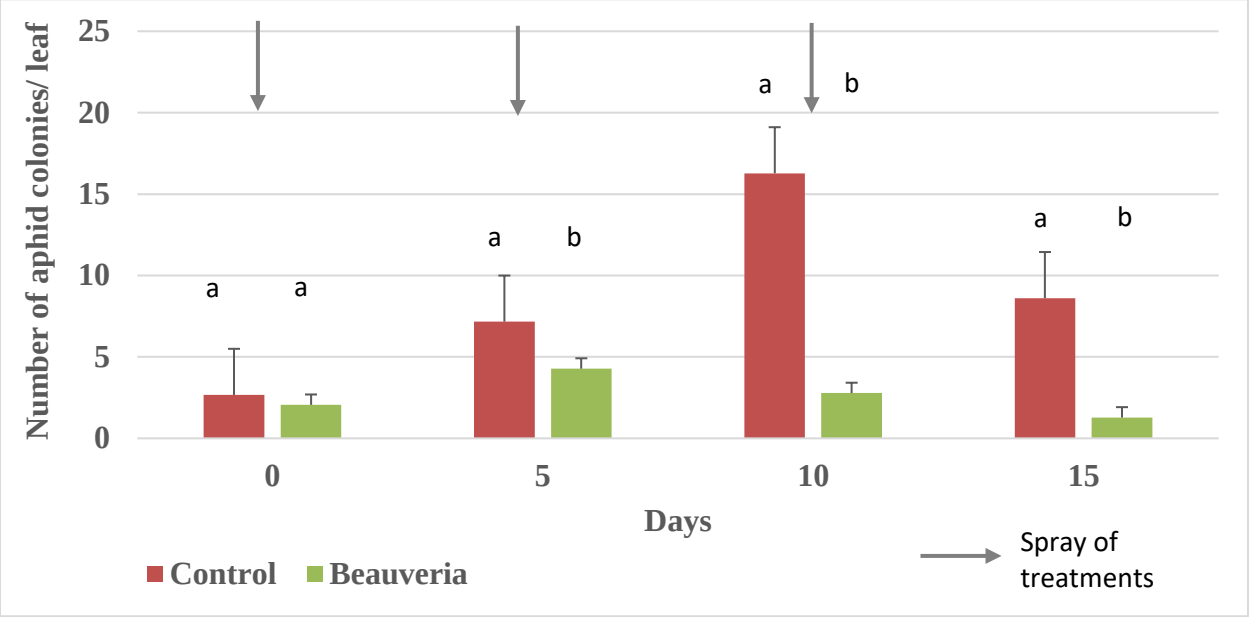

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Figure 26. Average number of aphid colonies/ leaf on cucumber plants in the oil control and Beauveria treatments, over a period of 15 days. Different letters indicate statistically significant difference ($P<0.05$).

The local *B pseudobassiana* strain provided the same level of control reported by Filho et al. (2011) for a commercial strain of *B. bassiana*. Aqueous conidial suspensions (0.01% Tween 80 + 0.01% v/v Agral) of three fungal isolates were sprayed twice at different dates, each with 2.0 x 10^9 viable conidia per potted plant using screened cages. The number of nymphs and adults of *M. persicae* per leaf was significantly reduced in plots treated with isolates CG 864 and PL 63, with control efficiency ranging from 57% to 60%. Further field trials using screened cages with isolate CG 864 formulated as oil dispersion reduced the aphid population by 85-87% as compared to the control, whereas a 71% reduction was seen in plants treated with the aqueous conidial suspension 20 days following the first spray. The results showed that the formulated *Beauveria bassiana*-based mycoinsecticides was effective against *Myzus persicae*, infesting cabbage under field conditions.

b. Whitefly control

Three days after the release of whiteflies, the first spray with *Beauveria* or oil took place (day 0) targeting the egg stage (Fig. 27). Five days later, there was 14.33 and 2.83 whitefly nymphs / leaf in the control and *Beauveria* treatments, respectively (80% reduction as compared to the control) and the second spray took place. Five days later (day 10), the number of whitefly nymphs decreased in both treatments to reach 6.94 and 0.83 nymph/ leaf, respectively (88% reduction over the control). There was a significant difference in the number of whiteflies between the treatments (P=0.006). This highlights the efficacy of *Beauveria* treatment, in managing the population of whitefly population. In addition to that, there was a significant difference in the days*treatment (P=0.03). The fungus was able to infect the egg and nymph stages of the whiteflies.

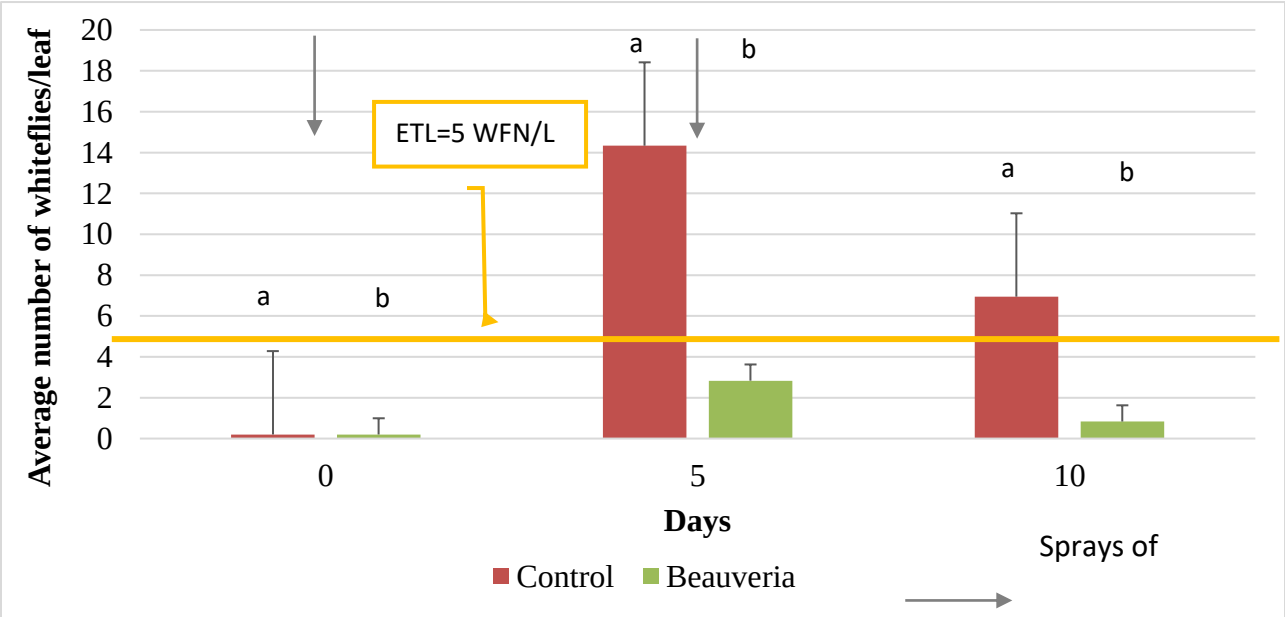

Figure 27. Average number of whitefly nymphs/ leaf on cucumber plants in the control and *Beauveria* treatments, over a period of 10 days. ETL = economic threshold level. WFN/L=Whitefly nymph/ leaf. Different letters indicate statistically significant difference (P<0.05).

The local isolate of *B. pseudobassiana* seems to have a better efficacy against whiteflies than reported for *B. bassiana* isolates. The experiment of Zafar *et al.* (2016), aimed at determining the effect of different isolates of entomopathogenic fungi, *B. bassiana* against different life stages of *B. tabaci* on multiple host plants *Gossypium hirsutum, Lycopersicum esculentum, Solanum melongena* and *Capsicum annum*. The results showed *B. bassiana* isolate (Bb-01) to be the most effective with LC50 value (2.4×10^7 conidia mL^{-1}) which caused highest mortality of eggs (65.30%) on *G. hirsutum* in comparison to other hosts, under laboratory conditions. In parallel, they concluded that the different plant hosts affected the egg and nymphs as the response was concentration dependent and mortality rates were highly significant. In addition, our results confirm the results of Baroudy *et al*. (2018) who proved that, the *B. pseudobassiana* isolated in Lebanon was found to be quite efficient for the management of *B. tabaci*. Sprays containing conidia suspensions of 10^7 conidia mL^{-1}caused around 75% mortality of the early growth stages: egg, crawler, second and third instar larvae. The addition of a surfactant such as corn oil improved the mortality level that reached 98% in the egg /crawler stage and 84% in the second and third instar larvae at a conidia concentration as low as 10^5 conidia mL^{-1}.

4. Evaluation of the efficacy of B. pseudobassiana against T. absoluta, under greenhouse conditions

a. Cages experiment

Under our experimental conditions (average temperature of 20°C, maximum temperature 25°C, and minimum temperature 18°C and 70% RH) eggs of *T. absoluta* hatched after 12 days of insect release The first spray of *Beauveria* was done 3 days after the insect release, on the day of egg counting (day 1), followed by the second spray after 8 days. The infected eggs (non-

hatched) turned, progressively, from milky-white to dark brown in color. Some of the infected eggs had successfully hatched; however, the emerged larvae were not able to burrow tunnels more than 5 mm in length. On the other hand, eggs in the control treatment successfully hatched, they did not show any sign of infection and the emerged larva was able to survive and burrow a tunnel exceeding 5 mm in length. All the eggs in the control treatment hatched (0% corrected mortality), while most of the eggs did not hatch in the *Beauveria* treatment with average mortality of 80%, after 2 sprays of the fungal solution. There was significant difference in the corrected mortality percentage between the control and *Beauveria* (P=0.000) (Fig. 28). This shows that *Beauveria* can infect *T. absoluta* eggs under greenhouse conditions when average temperature was 20 ℃ and significantly reduce infestation by this pest.

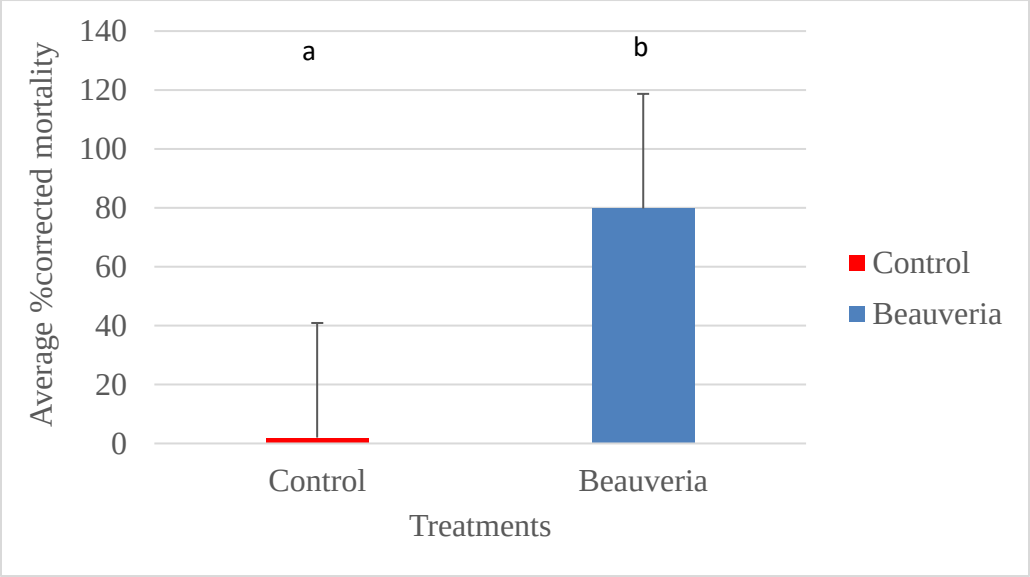

Figure 28. Efficacy of the *B. pseudobassiana* conidial suspension, against *T. absoluta* eggs in comparison to control treatment. A total of 57 eggs were studied. Average percent mortality (%) of three replicates. Different letters indicate statistically significant difference (P<0.05).

b. Greenhouse Experiment

The experiment was conducted under winter conditions, the average temperature was 14℃, maximum temperature 22℃, and minimum temperature 9℃, with a 75% RH. The eggs took 26 days to hatch under these conditions. Therefore, the first spray was done 3 days after insect release, on the day of egg counting (day 1), followed by the second spray after 13 days. The fungal conidia were able to infect *T. absoluta* eggs efficiently leading to 100% mortality of eggs after the application of 2 sprays, compared to 8.6% egg mortality in the control treatment, There was a statistical difference between both treatments (P= 0.000) (Fig. 29). In this trial, the percent of non-hatched eggs were calculated as %egg mortality rather than %corrected mortality, because there were 100% eggs non hatched in the *Beauveria* treatment. This shows that, *Beauveria* is highly effective against *T. absoluta* eggs under winter conditions.

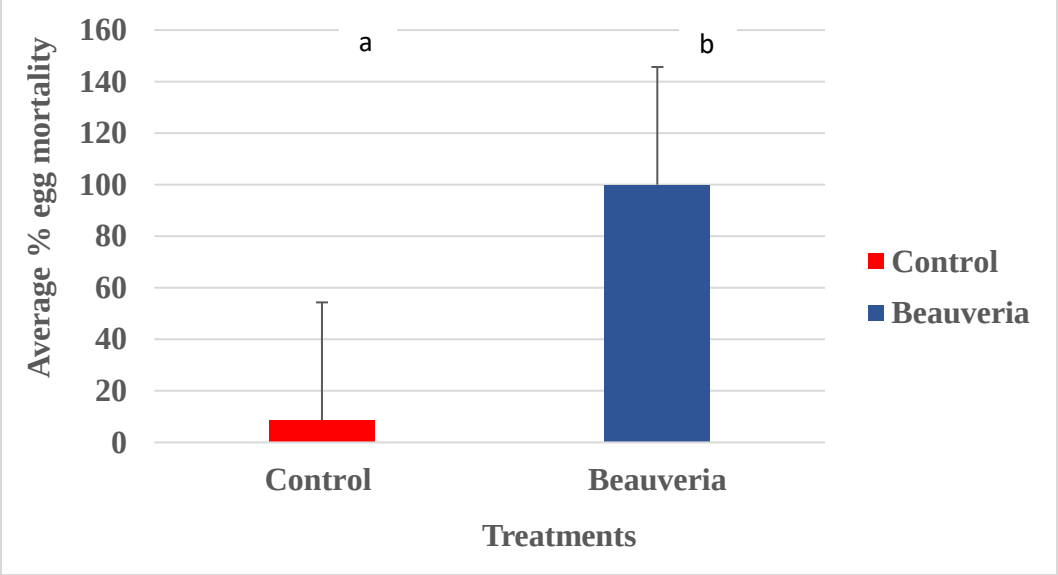

Figure 29. Efficacy of the *Beauveria pseudobassiana* conidial suspension, against *T. absoluta* eggs in comparison to control treatment under greenhouse conditions. A total of 258 eggs were studied. Average mortality (%) of three replicates. Different letters indicate statistically significant difference (P<0.05).

Our results show that the local *B. pseudobassiana* isolate is equally or more active against *T. absoluata* eggs as compared to the efficacy of other fungal isolates reported in the literature. Rodriguez et *al*. (2006) studied the pathogenicity of 64 *Metarhizium anisopliae* var. anisopliae and 70 *B. bassiana* isolates against tomato moth *T. absoluta* eggs, under laboratory conditions. The first evaluation was accomplished by spraying suspensions of 10^7 conidia mL^{-1} of each isolate directly on eggs, through a Potter tower. Mortality and conidia production on the eggs were significantly higher with the isolates *M. anisopliae* Qu-M558 and *B. bassiana* Qu-B911, Qu-B912 and Qu-B928. These isolates were newly evaluated using increasing conidia concentrations (10^6, 10^7,10^8 conidia mL^{-1}) of each of the five selected isolates. The isolates Qu-B912 and Qu-M558 produced the highest mortality percentages, 80 and 60%, respectively. Thus, the highest efficacies of these fungi, reported under laboratory conditions, are like the local isolate under greenhouse conditions.

B. Mass production and evaluation of the efficacy of *P. persimilis* for the management of *T. urticae*

1. Best rearing ratio of pest: predator for mass production

Figure 29 shows the change in spider mite population and in *P. persimilis population*, when 3 different spider mite: *Phytoseiulus* ratios were tested. The figure shows that there was a significant difference in the average number of spider mite population between treatments when different release ratios were applied (F=44.36, df=3, P=0.000). were tested. The figure shows that there was a significant difference in the average number of spider mite population between treatments when different release ratios were applied (F=44.36, df=3, P=0.000).

One week post the release of *Phytoseiulus*, the population started to increase with a significant difference between the treatments (F=22.90, df=3, P=0.000). The increase in predatory mite population was the fastest in the 40:1 treatment, where there was an increase by 10 predatory mites/ week, it was significantly 20:1 treatment (P=0.000). It was followed by the 60:1 treatment, where there was an increase by 5 predatory mites. The number of *Phytoseiulus* in 60:1 was not significantly different from the 20:1 (P=0.069), and 40:1 (P= 0.436). And the least increase was in the 20:1 treatment, where the population was increasing by 4 predatory mites/ week, which was significantly different from the control treatment only (P=0.017).

Figure 31 shows the available number of spider mites for every predatory mite. It aims at determining which release ratio will reach faster 3:1, the ratio used by commercial companies to deliver to farmers. One-week post *Phytoseiulus* release, 20:1 had 164.6 spider mites available for 1 *Phytoseiulus*, considered the highest population of spider mites. Then the population decreased sharply to reach 5.35:1 spider mite at week 4. The second highest population of spider mites recorded was 75 spider mites for 1 *Phytoseiulus* at 40:1. However, the available spider mites decreased to reach the lowest number of spider mites to *Phytoseiulus*, 5:1 at week 4. In 60:1 treatment, there was 68.2 spider mites for every predatory mite at week 1 and decreased to 16.57:1 spider mites: *Phytoseiulus* at week 4. This ratio of release was very low which did not allow the rapid decrease in pest population in a period of 4 weeks compared to 20:1 and 40:1. In all 3 treatments ratios, it took *Phytoseiulus* a lag phase of about 1 week to establish and start increasing. These data show that the 40:1 release rate is the most convenient ratio, it provides the highest number of predatory mites as well as fastest time to achieve the 3:1 ratio, according to figure 31, the ratio recommended to deliver to farmers for commercial use; according to the graph it needs 2 more days after week 4 to reach exactly 3:1 ratio

In conclusion, even though the number of *Phytoseiulus* reached after 4 weeks was not significantly different between 20:1 and 40:1, the 40:1 ratio gave higher production of *Phytoseiulus*. On the other hand, in the 60:1 ratio, the *Phytoseiulus* to spider mite population was low which lead to an increase in mite population resulting in plant damage. Thus, the most suitable release ratio 40:1, it was able to produce more *Phytoseiulus* without causing death of the plant, and reaching the 3:1 ratio, about 4 weeks post *Phytoseiulus* release.

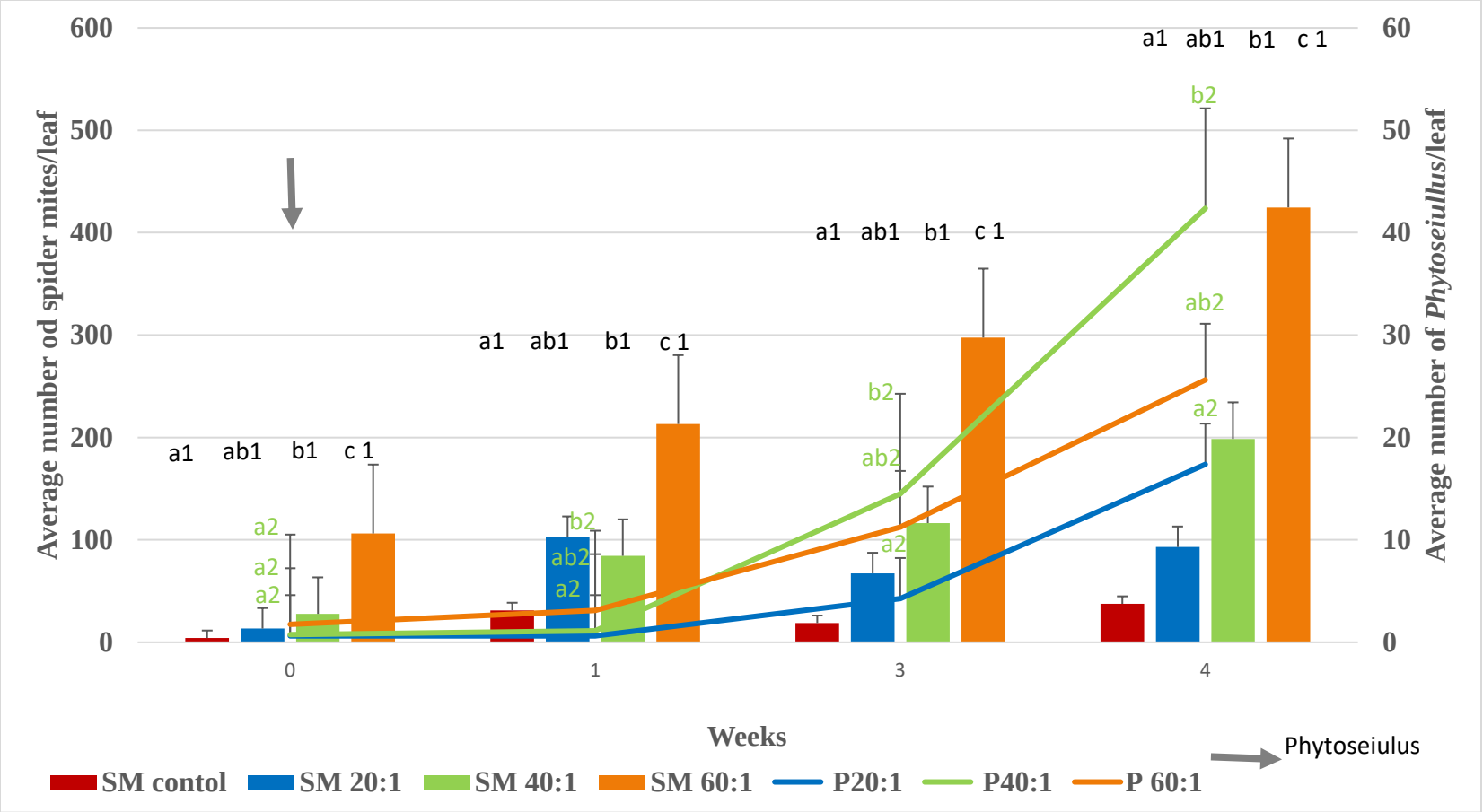

Figure 30.Population dynamics of spider mites and *Phytoseiulus* over time, weeks post *Phytoseiulus* introduction. Different letters indicate statistically significant differences according to Bonferroni's test (P≤0.05); letters in black refer to spider mite population, those in green refer to *Phytoseiulus* population. SM20:1= spider mites in 20:1 (spider mite/*Phytoseiulus*) treatment, P20:1= *Phytoseiulus* in 20:1 treatment. SM40:1= spider mites in 40:1 treatment, P40:1=*Phytoseiulus* in 40:1 treatment. SM60:1= spider mites in 60:1 treatment, P60:1=*Phytoseiulus* in 60:1 treatment.

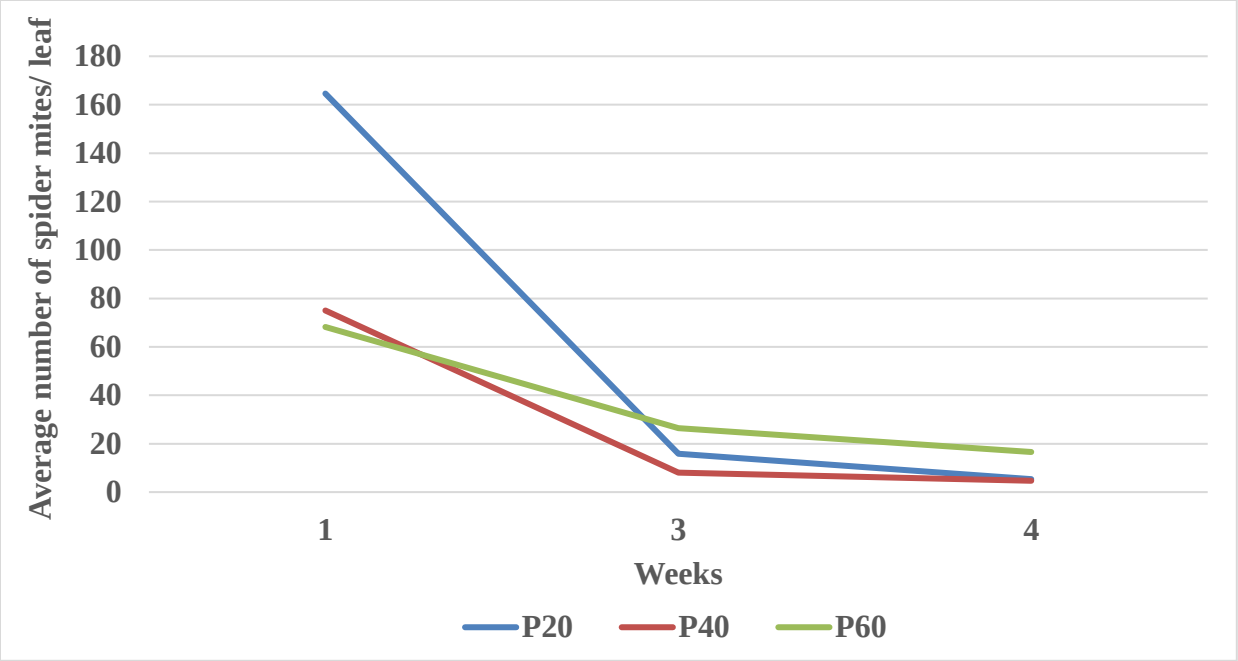

Figure 31. Average number of spider mites available for 1 *Phytoseiulus* in the three rates of release, at weeks post *Phytoseiulus* introduction.

2. *Efficacy of the local P. persimilis and B. pseudobassiana strains for the management of spider mites in a small greenhouse within an IPM strategy.*

Results of this preliminary experiment aiming at evaluation of the efficacy of *Phytoseiulus* in the management of spider mites and its possible integration with other control measures used in organic agriculture, such as oil sprays and *Beauveria* sp. used to control other arthropod pests.

In the control treatment, the number of spider mites doubled in one week to reach 300mite/ cucumber leaf (T average: 19±2℃, 86% RH) (Appendix III, Table III). However, the population of spider mites started to decrease**,** with the migration of *Phytoseiulus* from the treated plants into the control ones, 14 days post *Phytoseiulus* release**.** The spider mite population continued decreasing till week 3 when it reached 64.55 mite/ leaf in the presence of 4.66 predatory mite/ leaf, showing that the predatory mite searching ability is high and was able to

escape from the treated plants used in this trial (Fig. 32). In parallel, one week post the first spray with oil+ *Phytoseiulus* or *Phytoseiulus* + *Beauveria* the population of mites decreased by 40 % and 60 %, respectively. However, at 2 weeks post the first sprays and 1 week post the second sprays, the spider mite population decreased by 51% in the oil+ *Phytoseiulus* treatment and 87% in the *Phytoseiulus* + *Beauveria* treatment. There was significant difference in the average number of spider mites between the control and *Beauveria* treatments (P=0.04), however no significant difference in the number of spider mites in the oil and *Beauveria* treatments (P=0.069).

One week post sprays with oil or *Beauveria* , the population of *Phytoseiulus* increased from 14 to 19 predatory mite/ leaf in the oil treatment and from 14 to 16 predatory mite in the *Beauveria* treatment, showing that the sprays did not negatively affect the population of *Phytoseiulus*. This increase in *Phytoseiulus* population was accompanied by a decrease in the spider mite population. One week after the second spray, the spider mite population continued to decrease in the oil treatment and *Beauveria* treatments. At this point, the population of *Phytoseiulus* started to decrease in both treatments without a significant difference in both treatments. It decreased to reach 8.6 predatory mites/ leaf in oil treatment and 7.4 predatory mite/ leaf in the *Beauveria* treatment. This may not be correlated with a negative impact of oil or *Beauveria* on the *Phytoseiulus,* but it was rather correlated with decrease of the prey /food source.

These promising preliminary results prompted us to conduct a more detailed experiment to confirm these results.

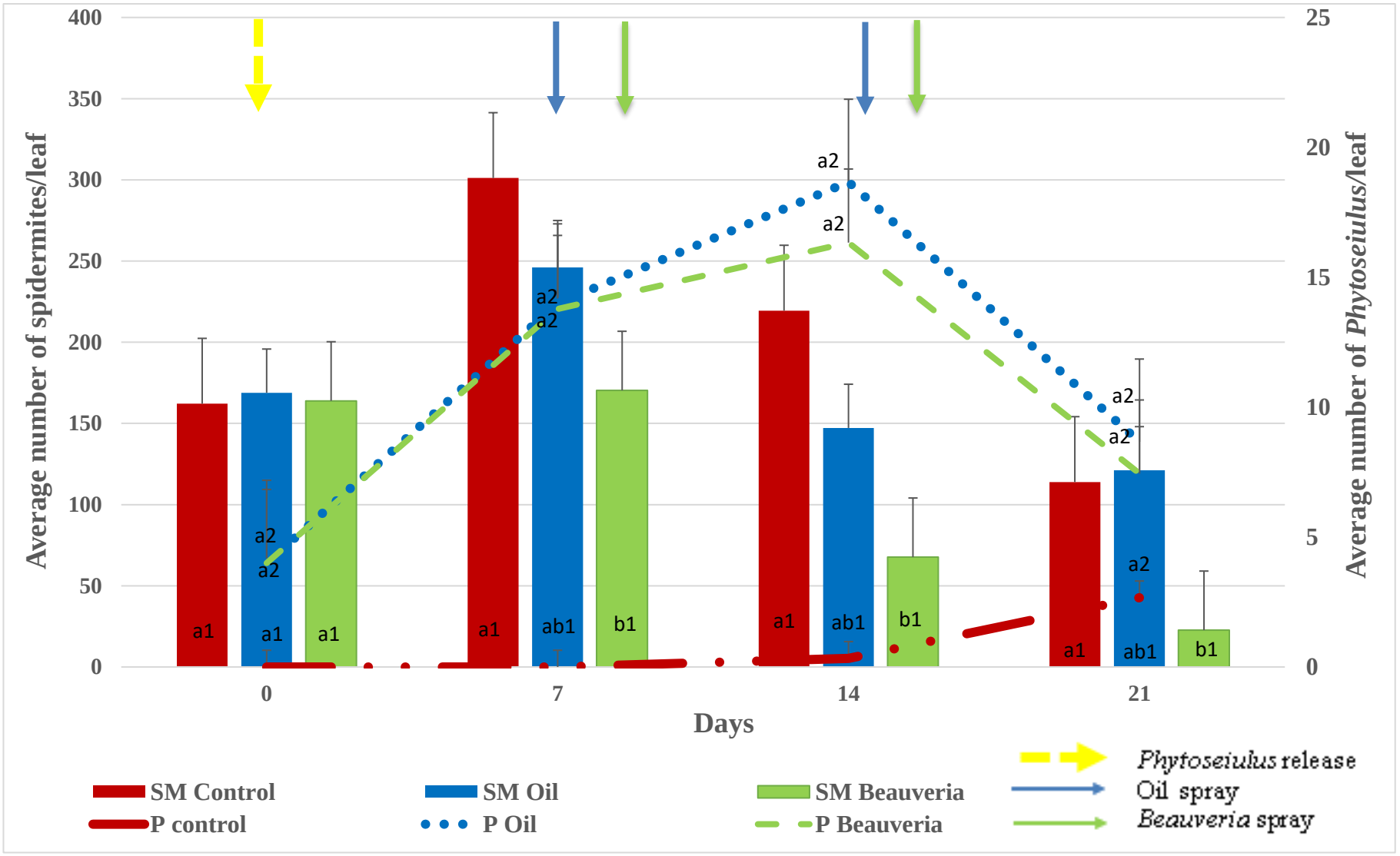

Figure 32.Population dynamics of spider mites and *Phytoseiulus* in weeks post inoculation of *Phytoseiulus*. Different letters indicate statistically significant differences according to Bonferroni's test (P≤0.05).

3. *Efficacy of the local* **P. persimilis strain** *for the management of spider mites in a small greenhouse within an IPM strategy in insect-proof cages*

Figure 33 shows that the number of spider mites was continuously increasing in the untreated control to reach 110 mites/ leaf at day 21. The spider mite population tripled in one week between day 14 and 21**,** under optimal climatic conditions for mite reproduction (T average: 23±2℃ and 67% RH) (Appendix III, Table IV). There was a significant difference in the average number of spider mites between the different treatments (F=34.695, df=4, P=0.000).

At day 14, one-week post application of the treatments, all treatments significantly reduced mite populations as compared to the control, except for the oil treatment. Then at day 21, 2 weeks post the second application of sprays, the number of spider mite increased by 53% in the oil treatment, as compared to day 14 but showed a control efficacy of 61% as compared to the control. However, the population of spider mites was reduced in the *Phytoseiulus+ Beauveria*, *Phytoseiulus* alone and *Beauveria alone* treatments, by 100%, 96% and 88%, respectively. There were no statistically significant differences between the latter 3 treatments ($P_{Phytoseiulus+Beauveria}$=1.00, $P_{Phytoseiulus}$=1.00, $P_{Beauveria}$=1.00).

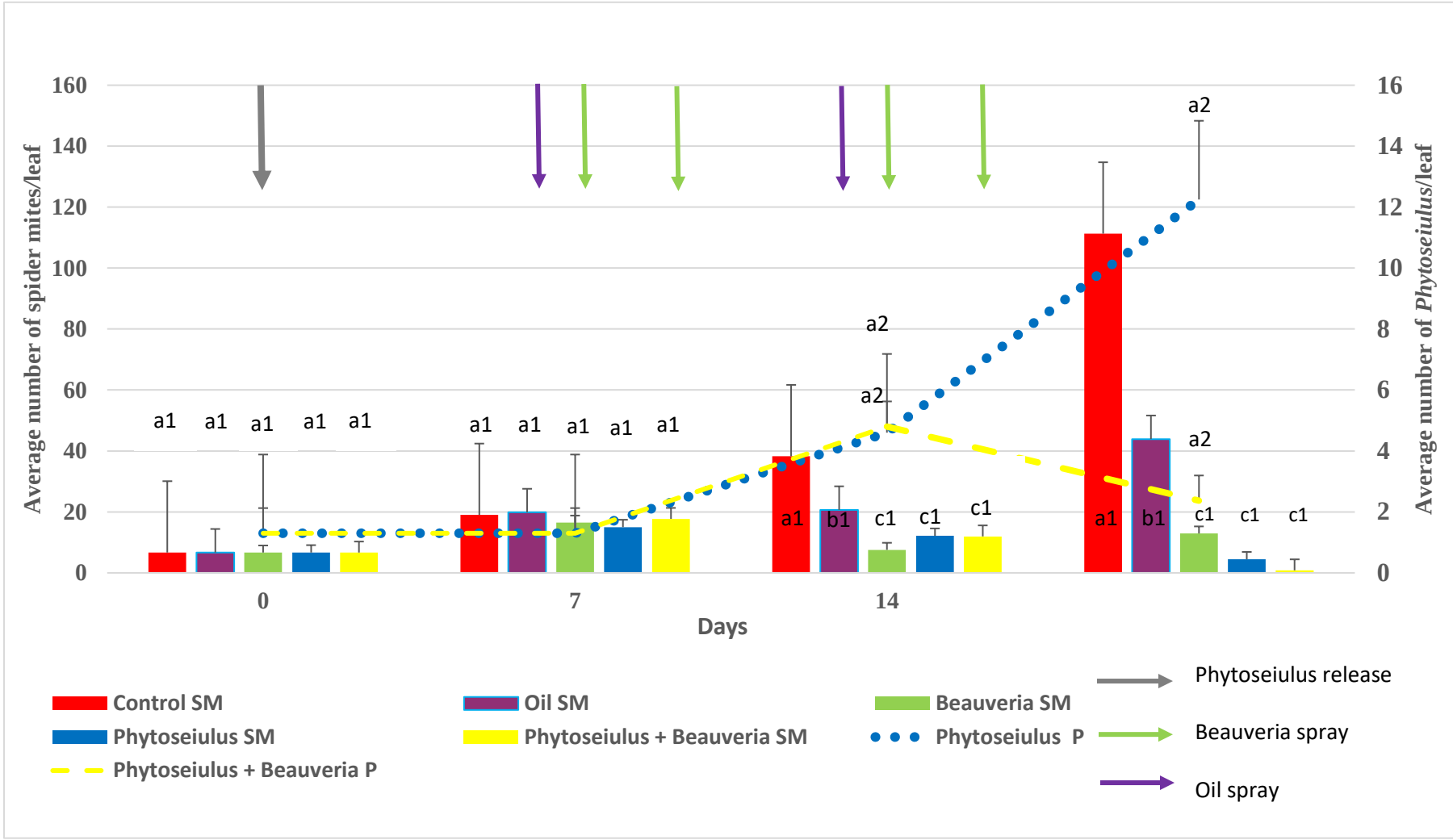

Figure 33. Average number of spider mites and *Phytoseiulus* in integrated pest management program. SM: spider mites, P: *Phytoseiulus.* Different letters indicate statistically significant differences according to Bonferroni's test (P≤0.05).

The number of *Phytoseiulus* was increasing *Phytoseiulus* alone and *Phytoseiulus* + *Beauveria* in treatments upon feeding on spider mites, thus reducing the population of the pest (Fig. 33). At day 14, the number of *Phytoseiulus* increased similarly in both treatments *Phytoseiulus* + *Beauveria* and *Phytoseiulus* alone. The number of *Phytoseiulus* increased from 1.3 to 4.8 predatory mites/ leaf in both treatments. This shows that the *Phytoseiulus* was not affected by the first spray of *Beauveria*. Then, at day 21 and after one week of the second spray of *Beauveria* solution, the number of *Phytoseiulus* was reduced in the *Phytoseiulus*+ *Beauveria* treatment to reach 2.3 predatory mite/leaf, while it increased in the *Phytoseiulus* treatment to reach 12.25 predatory mite/leaf, with a significant difference in the number of *Phytoseiulus* between the two treatments (P=0.174). Beyond this point, the number of *Phytoseiulus* decreased sharply in the *Phytoseiulus* treatment to reach 1.37, and zero *Phytoseiulus* were found in the *Phytoseiulus*+ *Beauveria* at day 23 (Figure 34). This sharp drop in Phytoseiulus population, between days 21and 23, may indicate that the difference in *Phytoseiulus* observed at day 21, may be due to reduction in the prey in the combined *Beauveria* + *Phytoseiulus* treatment, rather than due to the negative effect of *Beauveria* on *Phytoseiulus.*

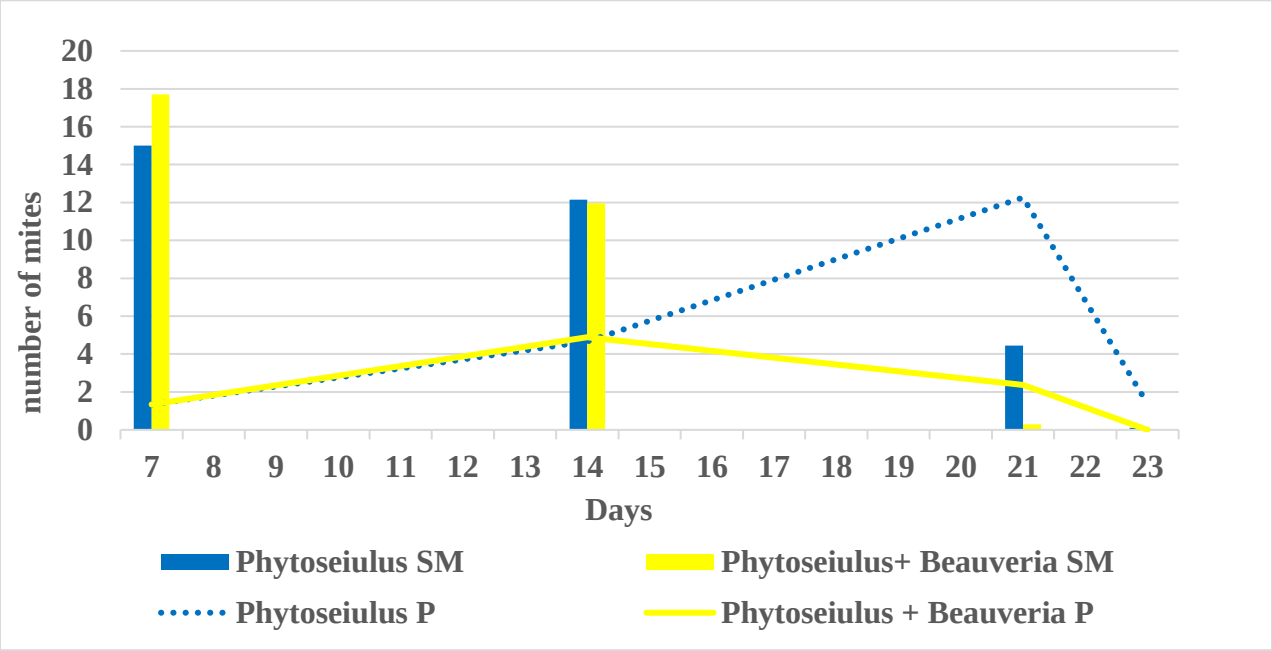

Figure 34. Average number of spider mites available as feed for *Phytoseiulus* population in the *Phytoseiulus* and *Phytoseiulus+ Beauveria* treatments. SM: spider mites, P: *Phytoseiulus*. Different letters indicate statistically significant differences according to Bonferroni's test ($P \leq 0.05$).

In conclusion, in the preliminary experiment, the sprays of *Beauveria* conidia suspension were able to control the population of spider mites (87% efficacy); however, two oil treatments at an interval of 7 days were moderately effective in reducing mite population on week later by 51%. And in the second trial, the three treatments *Phytoseiulus*, *Beauveria* and *Phytoseiulus* + *Beauveria* showed effective control of spider mites. Therefore, in an integrated pest management programs, it is recommended to use *Phytoseiulus* + *Beauveria* in case of infestation with multiple pests (such as whiteflies, aphids, etc.) or in case of high infestation levels of spider mites in greenhouses. The results of this experiment confirmed the outcomes of the preliminary study. The experiments proved that two sprays of *Beauveria* did not significantly affect the population of *Phytoseiulus* since there was no significant difference between the *Phytoseiulus* population in the oil and in the *Beauveria* treatments. While in the second experiment, a significantly lower *Phytoseiulus* population was observed one-week post-spray with the second *Beauveria*, spray

(day 21). However, this was correlated with a drop in the prey population rather with a negative effect of *Beauveria* since a rapid drop in the control *Phytoseiulus* population occurred at day 23.

Similar results were reported by Ullah and Li (2017) who observed a compatibility of *B. bassiana* and *P. persimilis* as biological control agents for control of *T. urticae* on potted bean plants, A single application of *B. bassiana* (1×10^8 conidia mL^{-1}) reduced the egg and adult populations of *T. urticae* initially, but mite populations rebounded again after few days. *P. persimilis* at the high release rate (5 preys: 1 predators) eliminated the pest population completely, while the low release rate (10:1) failed to control spider mites. The combined application of *B. bassiana* and low release rate of *P. persimilis* also successfully controlled *T. urticae* population ($P< 0.001$).

4. *Large scale field trials in commercial greenhouses to evaluate the efficacy of the local strain of P. persimilis in the management of T. urticae within an integrated pest management system.*

Results of this field trials aiming at determining the efficacy of the local *P. persimilis* for the control of *T. urticae* on cucumbers and peppers under Lebanese greenhouse conditions are presented in figures 35-41.

a. Control of spider mites on cucumbers

In the control greenhouse the farmer relied heavily on insecticides/acaricides to control arthropod pests on cucumber and pepper crops with a total of 14 pesticide sprays, each spray consisted of at least 3 active ingredients (Appendix II, Table II). Spider mite population was maintained below the economic threshold level of 2 mites/ leaf during spring (Fig. 35). However, with the increase in temperature during May-June the population of mites started to increase and exceeded the threshold level on June 5, 2019 (4.38 mite/ leaf). The population then increased to

reach an average of 53 spider mite/ leaf at the end of the growing season, effectively it increased 7x during the last week (June14-21). The farmer used Abamectin, Acetamiprid, Thiamethoxam and Pyridapen on June 11 and 14; however, no active ingredient was efficient in the control of mites. Therefore, the farmer decided to stop the production cycle on June 21, 2019.

In the IPM greenhouse, *P. persimilis* was released to suppress the mite population on cucumbers and peppers (Table 6) (Appendix II, Table III). At the early stage of the growing cycle, 10,000 *Phytoseiulus* adults (22.22 predatory mites/ m2) were released in the greenhouse for the protection against overwintering stages of spider mites. During most of the growing season, the spider mites and *Phytoseiulus* populations were almost negligible. During the first period of the growing season, the spider mites and *Phytoseiulus* populations were almost negligible, and the released predatory mite did not establish in the greenhouse for the absence of its prey. Table 2 shows the dates, the rates of release of *Phytoseiulus,* the average spider mites/ leaf and the ratio of spider mites to *Phytoseiulus* throughout the growing season. Then starting from May, hot spot treatments of *Phytoseiulus* were applied with the first symptoms of spider mite infestation. The spider mite infestation was concentrated in one side of the greenhouse, an estimated area of 250m^2 out of 450 m^2 (infested area + buffer zone). Beginning from May 22, the population of spider mites started to increase and exceeded the economic threshold by 3.08 mites/ leaf, on May 29, 2019. At this point *Phytoseiulus* started to establish in the greenhouse, feeding on spider mites. The spider mite's infestation took place on one side of the greenhouse (and reached the highest peak of 26.44 mite/ leaf on June 14. Therefore, *Phytoseiulus* releases were dense at the infested side, the release level was 4 predatory mites/m^2 at low infestation rates of spider mites and increased to reach 20 predatory mite/ m^2 on May 29. During this period, the ratio of spider mite: *Phytoseiulus* reached its highest level of 8.41 spider mites available for each

Phytoseiulus. Then, with the establishment of *Phytoseiulus* in the greenhouse the ratio of spider mites available for each *Phytoseiulus* was 3.41. During June, the spider mite population growth was faster than the predatory mites, the intervention with 36 predatory mite/ m2 took place. This inundative release of *Phytoseiulus*, in addition to the already present population, succeeded in managing the spider mite population and decreased it by 83% within one week, and a ratio of 0.25 spider mite for every *Phytoseiulus* was reached. The highest peak recorded for *Phytoseiulus* was 32.29 *Phytoseiulus*/ leaf on June 21.

Although spider mites were present in both greenhouses, the new growth of the cucumber leaves was greener and pest free in the IPM greenhouse compared to the control greenhouse (Fig. 36). In addition, Plants in the IPM greenhouse were healthy and vigorous, while in the control greenhouse the plants showed phytotoxicity symptoms and flower drop took place as a results of 14 sprays of insecticides/miticides, especially those applied during the hot weather in June (Fig. 36).

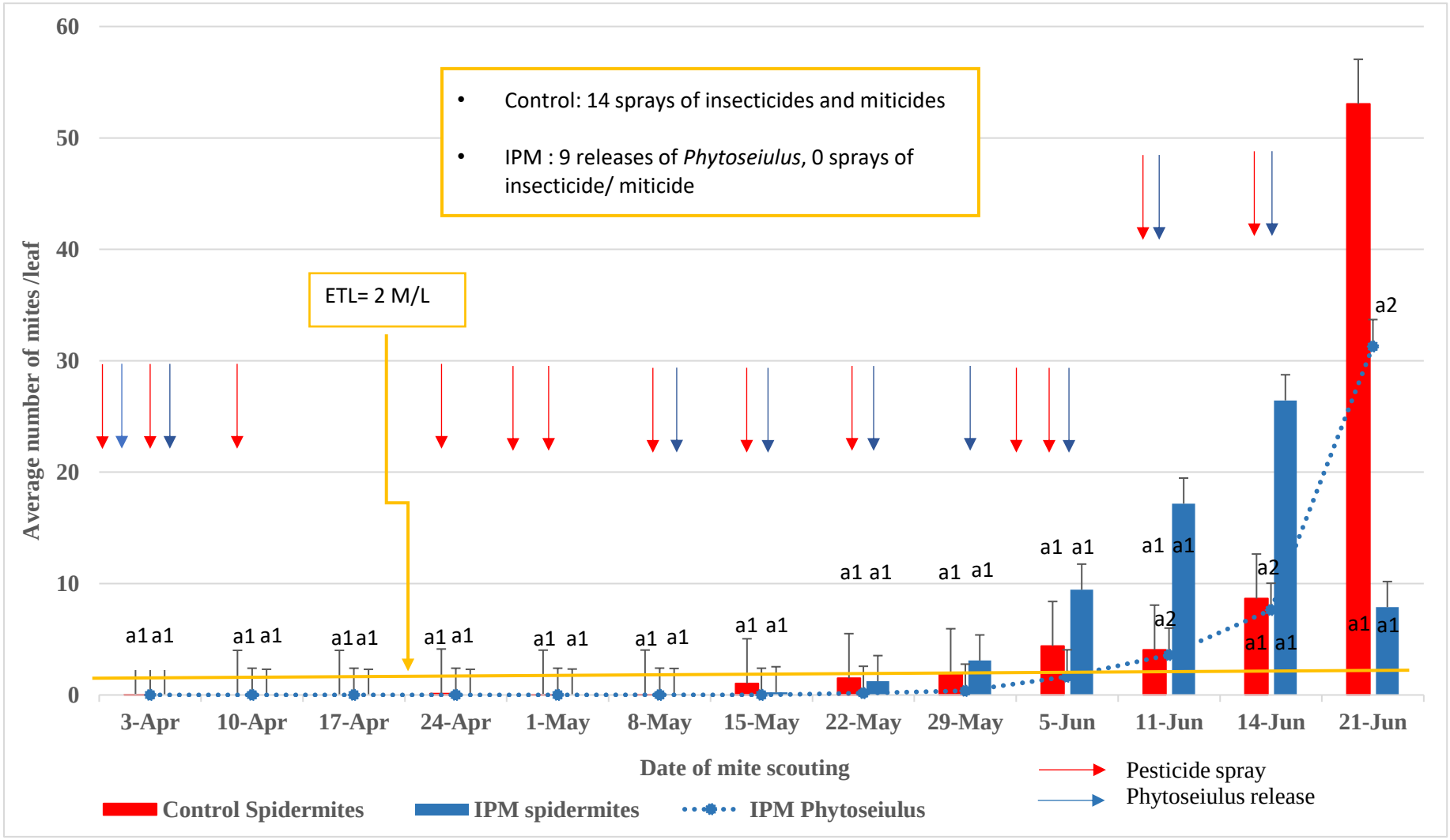

Figure 35. Average spider-mite and *Phytoseiulus* population recorded on cucumber leaves throughout the growing period in the control and IPM greenhouses. M/L: Spider-mites per leaf; ETL: Economic threshold.

Table 6. Release dates of *Phytoseiulus*, ratios of *Phytoseiulus*/m2 and the ratio of spider mites to *Phytoseiulus* on cucumbers and peppers.

Date	**Average spider mite/leaf**	**Released *Phytoseiulus*** **Total number**	***Phytoseiulus/m2***	**Ratio Spidermites/ *Phytoseiulus*** **On cucumber**	**Ratio Spidermites/ *Phytoseiulus*** **On pepper**
27-Mar	0	10000	22.22	0	0
3-Apr	0	200	0.88	0	1.476
10-Apr	0.0066	0	0	0	2.55
17-Apr	0	0	0	0	0.09
24-Apr	0	0	0	0	0.56
1-May	0.026	0	0	0	0
8-May	0.08	700	2.8	12	3
15-May	0.233	1000	4	0	0
22-May	1.233	1000	4	7.11	8.66
29-May	3.086	5000	20	8.41	15.25
5-Jun	9.44	500	2	5.71	7
11-Jun	17.16	2000	8	4.76	2.66
14-Jun	26.44	9000	36	3.46	0.26
21-Jun	7.88	0	0	0.25	-

Figure 36. Cucumber plants in the IPM and control greenhouses at the end of the experiment (June 21, 2019).

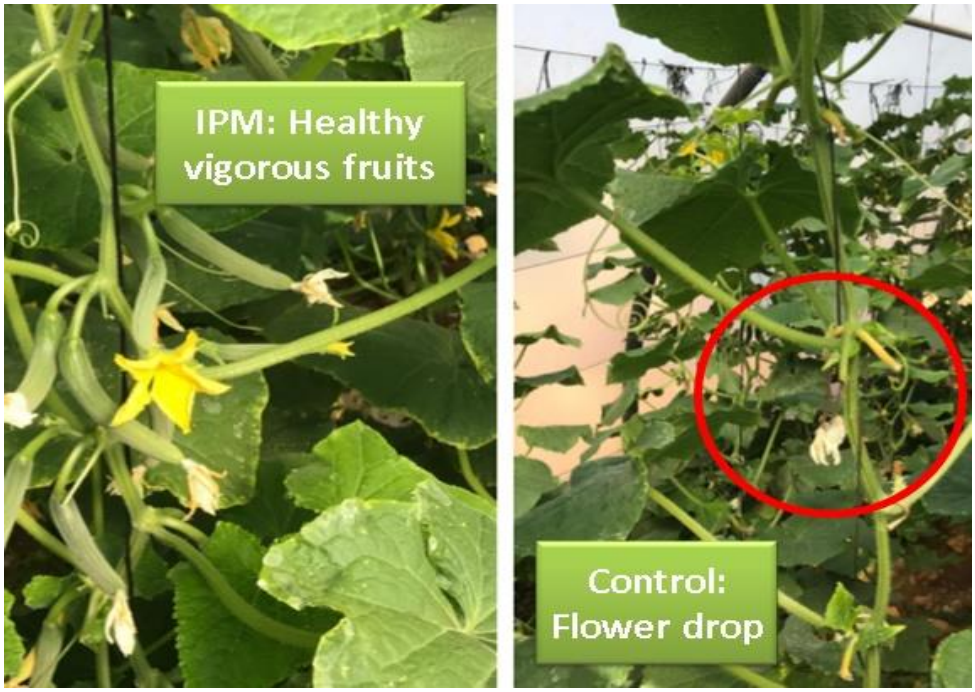

Figure 37. Healthy fruits in the IPM greenhouse compared to aborted fruits in the control greenhouses.

There was no significant difference in the number of spider mites between the two greenhouses (F=0.868, df=1, P=0.352). But there was a significant difference in the number of spider mites weeks*treatments (F=28.28, df=12, P=0.000). During May the average temperature was 25℃ and 65% RH, and during June the average temperature was 26 with 75% RH, however the maximum temperature recorded was fluctuating between 35 to 40℃ on multiple days (Appendix III, Table V and IV). The spider mite population exceeded the economic threshold, when the intrinsic rate of increase of spider mites (r = 0.91) was higher than that of the *Phytoseiulus* (r =0.74). The extreme temperature levels were favorable for the multiplication of spider mites, the population started to duplicate and then quadrupled from one week to another faster than that of the predatory mites. The intrinsic rate of spider mites according to the life table is 0.17 during the summer season, however throughout the experiment, the minimum rate was 0.43. This might be due to the migration of spider mites from outside the greenhouses that occurs in June. The release of *Phytoseiulus* in the greenhouse controlled the population of spider mites better than the pesticides, specially, when the spider mite population was high, by the end of June, showing that the local *Phytoseiulus* was active during the month of June and that it is heat tolerant.

b. Control of spider mites on peppers

On peppers, the spider mite population was fluctuating throughout the growing season (Fig. 38).

In the control greenhouse the spider mite population remained under control until June 2019. The population then, increased rapidly (r= 0.83) and exceeded the economic threshold with 2 peaks of 2.4 and 4.35 mite/ leaf, with the increase in the temperature on the 11^{th} and 14^{th} of

June. Multiple pesticide sprays were used to control the mite population, but the active ingredients did not prevent the outbreak of the population (Appendix II, Table 2).

In the IPM greenhouse, the mite population on pepper was managed by the releases of *Phytoseiulus* only. The mite population did not exceed the economic threshold level, in addition, there was 9.57 spider mites for every *Phytoseiulus*, on May 29. The highest peak recorded for the *Phytoseiulus* was 0.74 *Phytoseiulus*/ leaf on June 14, with a ratio of 0.5 spider mites for every *Phytoseiulus* (Table 6).

There was a significant difference in the number of mites between the 2 treatments (F=24.92, df=1, P=0.000) and in the number of spider mites in the weeks*treatments (F=7.26, df=11, P=0.000). This demonstrates that, the predatory mite was efficient in the control of spider mites on pepper, even at high temperature levels (maximum temperature above 32℃).

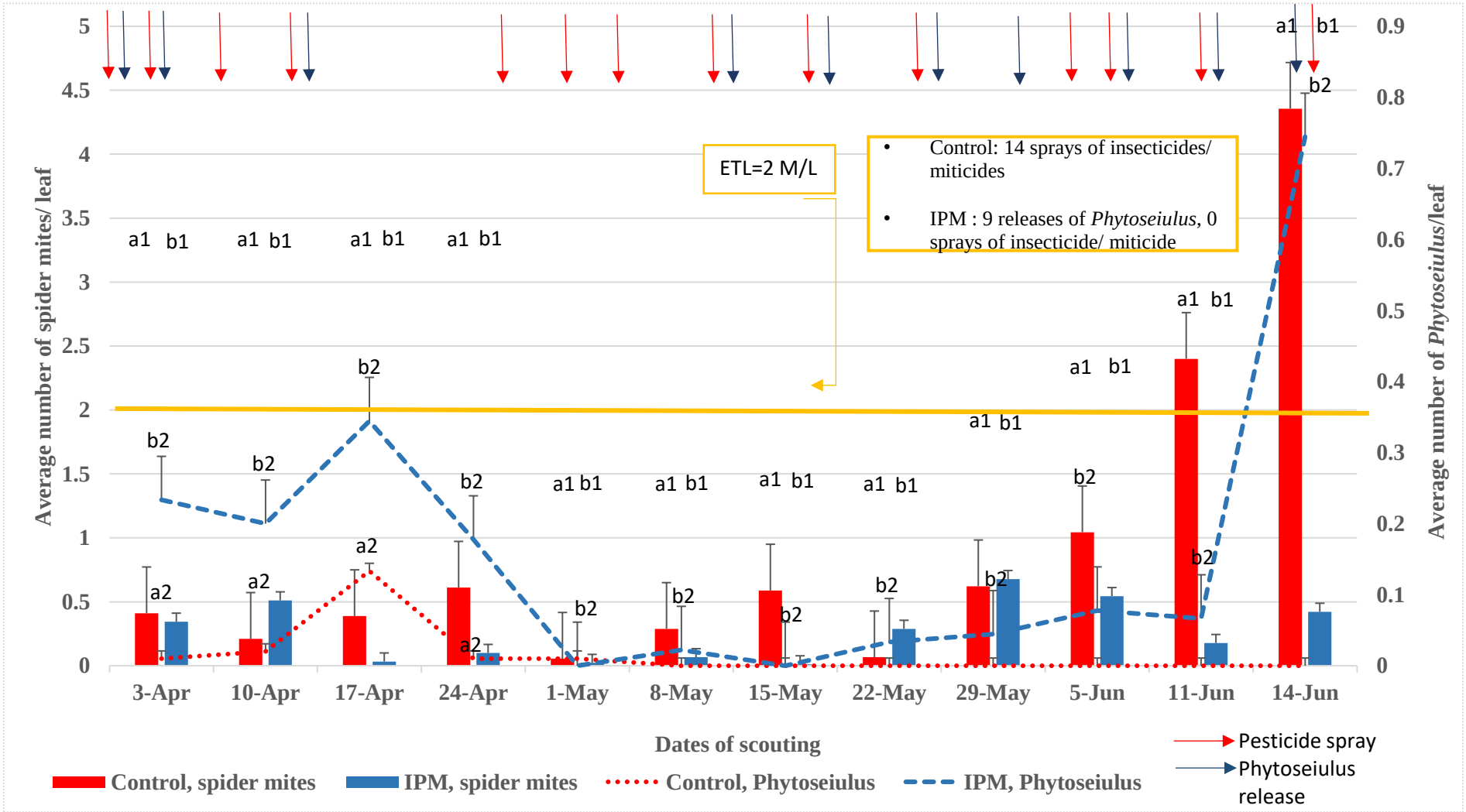

Figure 38. Averages of spider mite and *Phytoseiulus* populations in the IPM greenhouse compared to those in the control greenhouse, on pepper. M/L: Spider-mites per leaf; ETL: Economic threshold

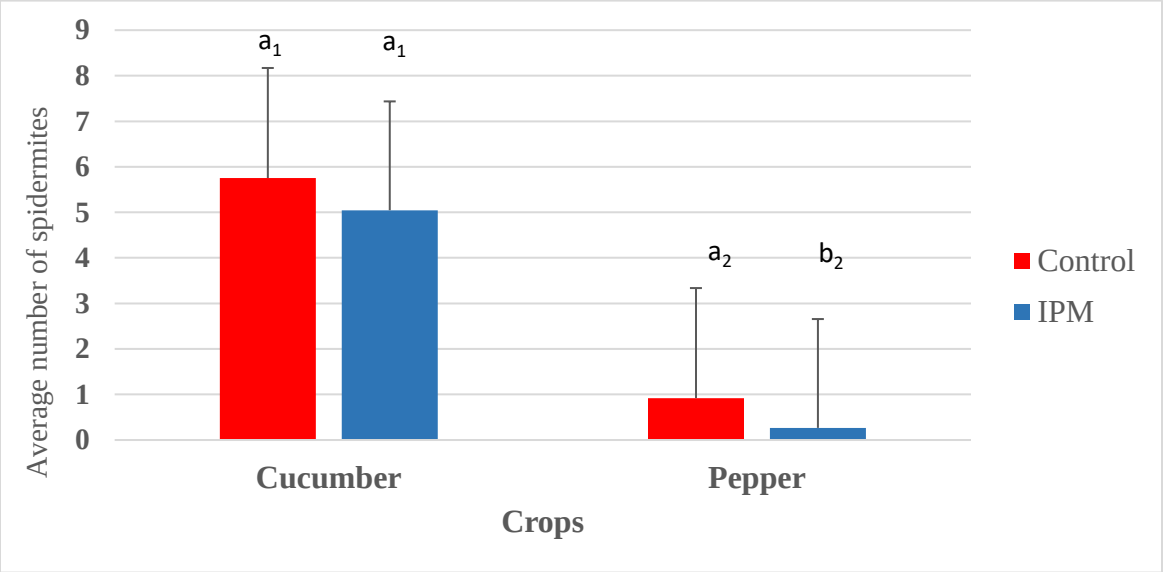

Figure 39. Averages of spider mite population over the growing season in the IPM greenhouse compared to those in the control greenhouse, on cucumbers and pepper crops. Different letters indicate statistically significant difference ($P<0.05$).

In conclusion, the local strain of *Phytoseiulus* was able to establish in the greenhouse and resist extreme climatic conditions prevailing in Lebanon during June and showed a high efficacy for the management of spider mites in greenhouse cucumbers and peppers grown during the spring/early summer season(Fig. 39). However, special attention should be paid to monitoring during the period following May 15. During this period, the average temperature was 25℃ and the relative humidity was 65%, however the maximum temperature reached 35 and 40℃ on several days, these conditions were very favorable for reproduction and multiplication of the spider mite population. For that reason, in warm weather, when there is about 1 spider mite/leaf, introductions of *Phytoseiulus* should occur at about 12.5 predatory mites/m^2 /week or 25 predatory mites/m^2 at biweekly intervals, in order to maintain a ratio of spider mites: *Phytoseiulus* should be maintained below 4:1 on cucumbers and 8:1 on peppers.

c. Control of aphids on peppers

Aphids were rarely found in the greenhouses; cucumber plants were free of aphids; they were scouted on pepper plants only.

In the control greenhouse, the aphids were absent throughout the growing season, except for 2 weeks during May. The highest peak of aphid population was 1.35 aphid colonies/ pepper leaf (Fig. 40), and it did not exceed the economic threshold level. The farmer was able to control the aphid population by the broad-spectrum of pesticides used. During these 2 weeks the sprayed active ingredients were: Abamectin, Acetamiprid, Thiamethoxam and Tolfenpyrad.

In the IPM greenhouse, the aphid population was fluctuating below the economic threshold, where 2 peaks were recorded. 0.53 and 0.23 aphid colonies/ leaf on May 1 and June 11, respectively. Three hot spot sprays of *Beauveria* conidia suspension were able to control the aphid population before their spread.

It is worth mentioning that shortly after the aphid peak and in in parallel to the 3rd *Beauveria* spray, mummified aphids were scouted on the pepper plants starting from May 8 (Fig. 40). This means that, in the absence of toxic pesticide sprays/residues, natural introduction of the aphid parasitoid wasp, *Aphidius colemani* took place. *A. colemani* was able to manage the aphid population without causing aphid outbreak and no further sprays of *Beauveria* solution were applied. In addition to *A. colemani*, natural introduction of the lace wing *Chrysoperla carnea* was observed in the IPM greenhouse during June 2019 (Fig. 42, Fig. 43)

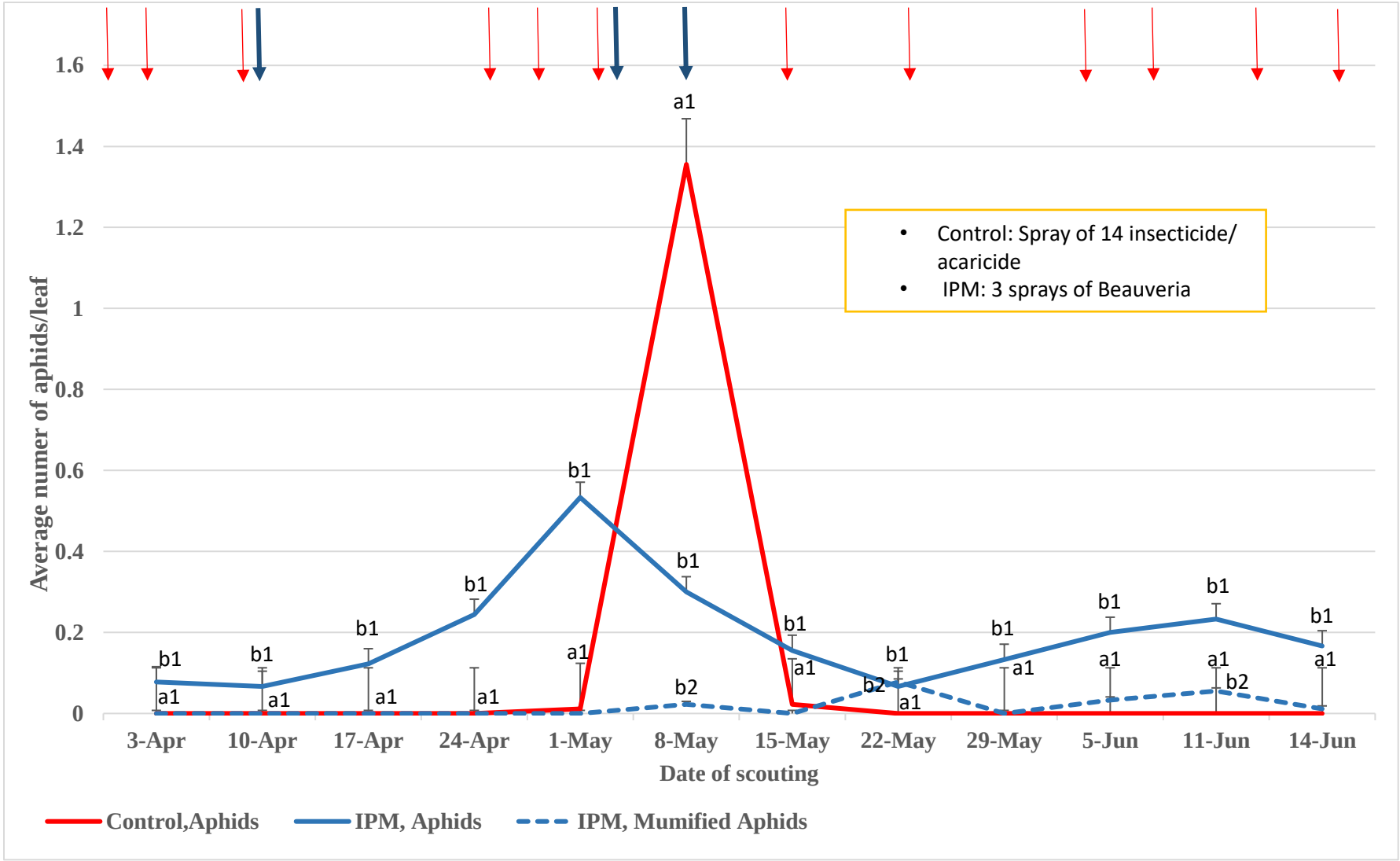

Figure 40. Averages of aphid and mummified aphid populations in the IPM greenhouse compared to those in the control greenhouse, on peppers.

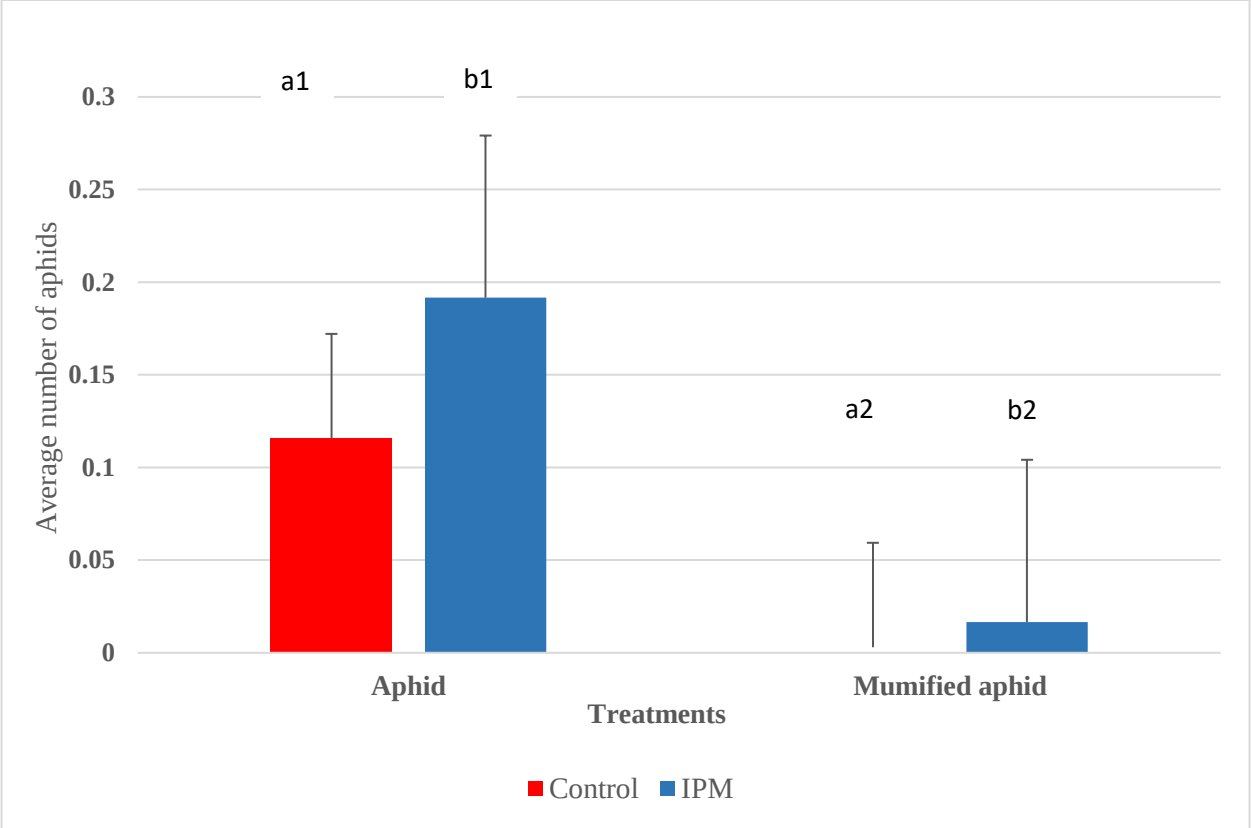

Figure 41. Averages of aphid population and mummified aphid in the IPM greenhouse compared to those in the control greenhouse, on pepper. Different letters indicate statistically significant differences according to Bonferroni's test (P≤0.05).

Although aphid population was under control in both greenhouses, there was significant difference in the average number of aphids between the control and IPM greenhouse (F=33.91, df=1, P=0.000) (Fig. 41). The population of aphids was higher in the IPM greenhouse, with an average of 0.19 aphid/ leaf. The *A. colemani* was maintaining the aphid population under control without causing collapse in the aphid population. No mummified aphids were present in the control greenhouse. This shows that the biological control agents *B. pseudobassiana* and *A. colemani* were able to manage the aphid population and that in the presence of the three introduced natural enemies, the other local natural enemies were also able to establish naturally.

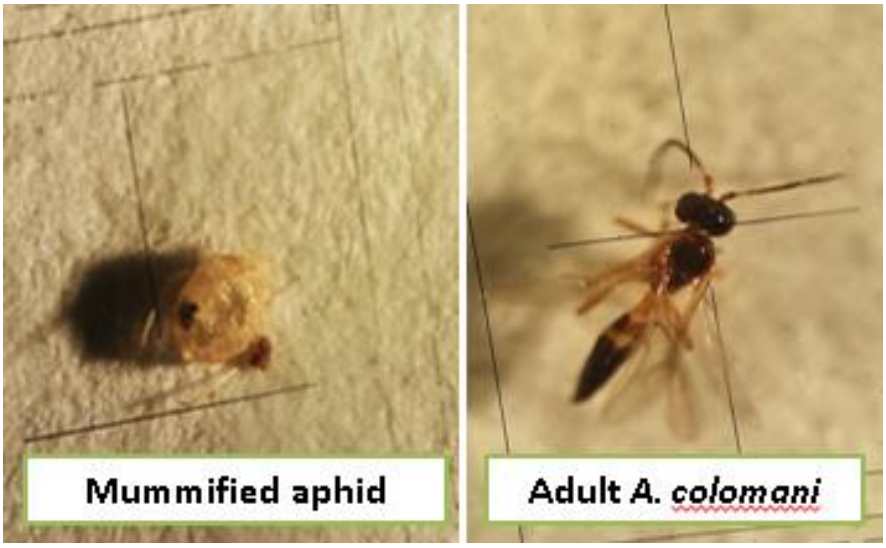

Figure 42. Mummified aphid and emerged *A. colemani* out of the aphid collected from the IPM greenhouse

Figure 43. *Chrysoperla carnea* adult collected from the IPM greenhouse

In conclusion, the efficacy of the local strain of *P. Persimilis* against spider mites was first observed in small scale trials, was confirmed in large scale trials in commercial size greenhouses on two crops, pepper and cucumber. Our results with the local strain are in agreement with Zhang et al (2003), who reported that in the presence of predators, spider mites showed an initially slow (<50%) increase followed by a decline concurrent with a rapid increase of predators. Spider mites were nearly eliminated within 5 weeks of the introduction of the predators. In Lebanon, particular attention should be paid to increase *Phytoseiulus* release rates staring from the end of May for two reasons: first the temperature would be very suitable for spider mite reproduction and second, mite migration from adjacent areas into the greenhouse during this period should be accounted for.

Yanar et al., (2019) showed the effectiveness of the predatory mite, *P. persimilis* as a suppressive agent of the two-spotted spider mite, on greenhouse cucumber at predator: prey release ratios of 1:5, 1:15, and 1:30. Releases at each predator: prey ratio were made at 30 *T. urticae* densities per leaf. In control treatments without predatory mite and acaricide application, population of *T. urticae* was constantly increased and reached 140 active forms/leaf in August. Subsequently the population decreased when the plants died. At ratios of 1:5, *P. persimilis* reached 8 active forms/leaf while *T. urticae* populations reached 11 active forms/leaf. At ratios of 1:15 *P. persimilis* population increased (3.4 active form per leaf) and *T. urticae* population decreased (1.6 active forms per leaf) in September and in the 1:30, the *P. persimilis* population was 20 active forms per leaf, compared to 42 spider mite active form/ leaf. Plant damage also was significantly reduced at these densities. *P. persimilis* population decreased when prey population decreased. The results of their experiment are somewhat in agreement with those of

our experiment, they proved that *P. persimilis* was able to provide effective control of *T. urticae* on a greenhouse-grown cucumber at a moderately low predator:prey ratio (1:15).

Calin et al., (2017) studied the biological control of two-spotted spider mite on pepper and melon crops cultivated in tunnels, using *P. persimilis*. The attack of two-spotted spider mite began in the first decade of June. The degree of attack of two-spotted spider mite on pepper plants was ascendant reaching 17.5% in the second decade of September. The variants utilized were as follows: V1 - one release with 50,000/ha; V2 - one release with 100 thousand /ha; V3 – one release with 150 thousand /ha; V4 - Untreated control. The releases of *Phytoseiulus* have reduced the two-spotted spider mite degree of attack (DA%) on pepper in late August to 2.3% in V1, 2.1% in V2 and 1.7% in V3. In September, the DA% was below 1% in all the three variants. In the untreated control, the pest degree of attack level increased from 9.5% in the first decade of August, up from 17.5% in the 2nd decade of September, and then began to decline. This shows that the *P. persimilis* was effective in the control of spider mites on peppers, with the most efficient release ratio was 150000 predatory mite/ ha, equivalent to 15 predatory mites/ m^2.

d. Economic analysis

According to the farmer the total cost of pesticides sprayed in a 450m2 greenhouse is about 550$, which is equivalent to 1.22$/ m^2. On the other hand, the cost of "Spidex": a 500ml bottle containing 10,000 *Phytoseiulus* is 171$, without the delivery charges. In our trial we used 30,000 *Phytoseiulus*, so if the farmer wants to import this predator the cost would be 513$, equivalent to 1.11$/m2 excluding the delivery costs. In addition, 2 other natural enemies were used in the IPM greenhouse, *B. pseudobassaina* and *A. swierskii*. The costs of the biological control agents are shown in Appendix IV, Table I.

Therefore, the use of pesticides is less expensive to the farmer, but with considerable negative side effects on human and environmental health. On the other hand, the use of the more expensive biological control agents within an IPM strategy, will give better quality fruits suitable for export with higher prices ensuring better farmer income that may overcompensate for the high cost of natural enemies.

At present, ordering natural enemies from outside Lebanon is prohibited and in case it will be allowed, the pricing would be relatively high compared to the initial price in the country of origin due to the high shipping and customs costs. Furthermore, the natural enemies may suffer during shipping and may arrive with a low quality and reduced activity. The local mass production of natural enemies can reduce the cost by at least 50-60%, provide high quality of vigorous natural enemies and eliminate the shipment and customs costs (Appendix IV, Table I).

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Most greenhouse farmers in Lebanon rely heavily on pesticide sprays to control vegetable pests with significant negative impact on human and environmental health. However, to cope with the international standards of food safety and security, in addition to the alignment with trade agreements, it is highly recommended to emphasize the role of natural enemies as an alternative measure to the synthetic chemicals. Multiple success stories related to the high efficacy of the natural enemies, including *P. persimilis*, were reported in many European countries and some states in USA (Van-Lenteren et al., 2018). Alongside the mentioned predatory mite, the entomopathogenic fungi *B. bassiana* and *M. anisopliae* have proven to be efficient control agents of several insect pests including *T. absoluta* (Inanli et al., 2012). In Lebanon, mass production and application of natural enemies for the management of arthropod pests would represent a pioneering activity in the field of greenhouse production.

The laboratory tests focused on mass production trials of *B. pseudobassiana* conidia and evaluated four grain substrates at two different grain to water (W/V) ratios (1:1 and1:0.5). At 1:1 ratio, both burghul and oat produced significantly higher concentration of conidia compared to wheat and rice, producing $3x10^9$ and $2.33x10^9$ conidia/g, respectively. While at 1:0.5 ratio, no significant difference in the concentration of harvested conidia was recorded between the four grain substrates. The highest conidia concentration was also harvested from burghul and oat, they produced $1.1x10^9$ and $1.06\ x10^9$ conidia /g, respectively. Although the conidia harvested from 1:1 were higher, but the texture of grains after 2 weeks of incubation, was better when 1:0.5was used. Tests focused also on enhancing the conidia germination of the of *B.*

pseudobassiana by adding multiple additives and adjuvants. The results showed that addition of mannitol significantly improved the conidia germination, while the addition of 1% corn oil to the conidia suspension, gave the highest germination rate of conidia 93%. This was significantly higher than the control which gave 85% germination and the adjuvants 0.25%Luqsa (79%) and 0.25% Vegetable oil (76%). Therefore, the addition of 1% corn oil to the conidial suspension was used in the efficacy experiments.

Small scale greenhouse experiments focused on the evaluation of *B. pseudobassiana* as an alternative control measure for aphids, whiteflies, and tomato pin worm *T. absoluta*. Aphids infected with *Beauveria* showed a whitish mycelial growth out of their soft bodies. The fungal isolate was able to limit the aphid colonies to 1.2 colonies/ leaf compared to 8.6 colonies/ leaf in the control, after 3 sprays. The *Beauveria* conidia were also effective in infecting the egg stage of the whiteflies and preventing them from further development to the nymphal stage. On average there was only 0.8 whitefly nymphs/ leaf in the *Beauveria* treatment, while there was 6.8 nymphs/ leaf in the control treatment. The number of aphid colonies and whitefly nymphs were significantly lower when the *Beauveria* conidia were sprayed. In parallel, the eggs of *T. absoluta* turned dark brown in color when treated with fungal conidia, some of the infected eggs had successfully hatched; however, the emerged larva was not able to burrow tunnels more than 5 mm in length. After two consecutive sprays, the egg mortality was 80% in the *Beauveria* treatment at average temperature 20℃, while all the eggs were able to hatch in the control treatment (0% mortality). In a second trial, conducted in winter season at an average temperature 14℃, two sprays with *Beauveria,* the first spray was done directly after egg laying and followed by a second spray, 8 days later, resulted in 100% egg mortality, while the egg mortality was only 9% in the control treatment.

For the mass production of *Phytoseiulus*, the attempt was to get the highest number of *P. persimilis* and to reach the ratio of 3 spider mites for every predatory mite, at the end of every production cycle. This ratio is perfect for the *Phytoseiulus* release in the field, since the predatory mites have enough prey to feed on during the transfer period, and the low number of preys will not lead to pest outbreak. For that reason, three different ratios of spider mites to *Phytoseiulus* were applied, 20:1, 40:1, 60:1. The highest rate of *Phytoseiulus* release (20:1), produced 4 predatory mites/ week and the lowest release rate (60:1) produced 5 predatory mites/ week and did not cope with spider mite population which damaged the plants. However, 10 predatory mites/ week were produced at 40:1 release ratio. This shows that the 40:1 ratio was the fastest in producing *Phytoseiulus* without damaging the plant. This also sheds light on the progressive change in the number of its progeny in relation to prey density, known as numerical response (Solomon, 1949). The efficiency of conversion of ingested food (ECI) is higher at low prey density, meaning that the females invest most of their energy in egg production and, in the process, invest less in maintenance and metabolic activities (Carrillo and Peña, 2012).

Small scale greenhouse experiments were conducted to determine the efficacy of the local strain of *P. persimilis* against *T. urticae* and its compatibility with *Beauveria* sprays intended for aphid and whitefly control within an integrated pest management (IPM) program on cucumbers. In the preliminary experiment, the treatments were 10^8 conidia mL^{-1} + 1% corn oil + 0.01% Tween-20 + *Phytoseiulus*, 1% corn oil + 0.01% Tween-20 + *Phytoseiulus*, and the untreated control. The results showed that, after 7 days of *Phytoseiulus* release, the spider mite population increased in the 3 treatments, with a significant difference in the mite population in the *Beauveria* treatment compared to the control, while no significant difference in the number of mites between the oil and control treatments. After the application of 2 sprays, the spider mite

population decreased by 87% in the *Beauveria* treatment, while it was reduced by 51% in the oil treatment, there was a significant difference between the 2 treatments. In the second trial, the experiment consisted of five treatments, 10^8 conidia/ml + 1% corn oil + 0.01% Tween-20 + *Phytoseiulus*, 10^8 conidia/ml + 1% corn oil + 0.01% Tween-20, *Phytoseiulus*, 1% corn oil alone in addition to the untreated control treatment. The spider mite population continued to increase until 7 days after of *Phytoseiulus* release, without a significant difference in the number of spider mites between the different treatments. However, by day 21, and after the 2 sprays of *Beauveria*, the number of spider mites decreased in the *Beauveria* treatment, *Phytoseiulus* treatment and in the combined *Phytoseiulus* + *Beauveria* by 88%, 96% and 100 percent, respectively. Noting that on day 14, the *Phytoseiulus* population increased equally in *Phytoseiulus* and the *Phytoseiulus*+ *Beauveria* treatments. But, one week later and after the 2nd *Beauveria* spray (day 21), the population of *Phytoseiulus* decreased significantly in the combined *Phytoseiulus* + *Beauveria* treatment as compared to the *Phytoseiulus* treatment alone. However, this difference was mainly correlated with the rapid shortage of prey that occurred in the combined treatment and may have led to the cannibalism phenomenon. This was deduced from the sharp drop in *Phytoseiulus* population that occurred in the *Phytoseiulus* treatment 2 days later, at day 23. In conclusion, these two experiments showed that the local strain of *P. persimilis* was highly effective against spider mites and that its use is compatible with the use of the local isolate of *B. pseudobassiana*, and therefore, these two biological control agents can be used together within an IPM program to broaden the spectrum of pest control and/or to enhance efficacy against spider mite.

To validate the results obtained in small scale trials, large scale trials in commercial size greenhouses were conducted at Kfarmashoun - Jbeil, aimed at assessing the efficacy of the locally collected *B. pseudobassiana* and *P. persimilis* against aphids and the two-spotted spider

mites. Whiteflies and thrips were also monitored and controlled by *Amblyseius swirsikii,* however the results are presented in another thesis. The experiment was conducted between April and June 2019, throughout the growing period the average temperature was 23 ±2°C and 67% RH, with increase in the maximum temperature as we approach the summer season.

In the control greenhouse, the farmer followed his normal plant protection practices, the pre-transplanting measures implemented and the application of 14 sprays of broad-spectrum insecticides/acaricides have successfully reduced the whiteflies and thrips pest populations on cucumber and pepper. On the other hand, the population of two-spotted spider mites, on cucumber, remained below the economic threshold level (2mites/leaf), early in the growing season. However, the increase in average temperature in May and June with a maximum temperature reaching over 36°C, the spider mite population increased rapidly and exceeded the threshold level (4.3 mites/leaf) on June 5, 8.3 mites/leaf on June 14 and 53 mites/leaf on June 21, a 6 fold increase in population within one week, forcing the farmer to remove the crop. This significant increase in the pest population was mainly due to suitable environmental conditions, resistance of mites to the applied acaricides and probably to the migration of mites into the greenhouse from adjacent fields. On the contrary, the population of spider mites on peppers was more controllable. The population remained below the economic threshold level throughout most of the growing season, except for 2 peaks during June 11 and June 14 when the spider mite population exceeded the threshold level and reached 2.4 and 4.3 mites/ leaf, respectively. *Phytoseiulus* adults were observed in the control greenhouse only once April. The mites either migrated searching for feed (low spider mites in the IPM greenhouse), or they were transmitted by the workers when moving between the greenhouses. Aphid colonies were also observed on peppers for one week during May, the broad-spectrum pesticides used eliminated them rapidly.

The plant health was negatively affected by the spraying program during the hot weather in May and June, the leaves were burned, and flower drop took place.

In the IPM greenhouse, *Phytoseiulus* was released for the control of spider mites and *Beauveria* was sprayed for the control of aphids. The spider mite population was relatively low and below the economic threshold level during April. Then, with the slight increase in the spider mite population, during May, hot spot releases of *Phytoseiulus* took place at the rate of 2.4 and 4 predatory mites/m2, The infestation of spider mites was localized on one side of the greenhouse, so the release pressure was focused on that area in addition to a buffer zone between the infested side and the clean one. On May 29, the spider mite population exceeded the ETL, therefore, 20 *Phytoseiulus*/ m2 were released in the infested area, there were 8.4 spider mites for every *Phytoseiulus*. However, with the increase in maximum temperature (Tmax=36℃), the climatic conditions were optimal for development of high numbers of spider mites. During this period, a low release rate of *Phytoseiulus* took place due to the low numbers of *Phytoseiulus* harvested from the rearing system. Despite that, the spider mite ratio decreased from 5.7:1 on June 5 to reach 4.7:1, on June 11. Beyond this point, the spider mite population reached the highest peak of 26.44 mites/leaf on June 14. The release of 36predatory mites/ m2 then took place and led to the sharp decline in the spider mite population to reach 7.88 mite/leaf on June 21. This means that the effective introduction of *Phytoseiulus* led to the decrease in spider mite population by 70%, in one week; and the ratio of spider mites: *Phytoseiulus* was reduced from 3.46 to 0.25: 1. The new growth of cucumber leaves was free of mites, and the plants were more vigorous than in the control greenhouse. This illustrates the efficacy of *Phytoseiulus* in controlling spider mite infestation when applied at the proper time and date, and even at elevated temperatures, suggesting that this local isolate is somewhat heat tolerant. Release program of *Phytoseiulus*

during the warm season should rely on weekly and/or biweekly releases of predatory mites to avoid the outbreak of spider mites based on monitoring results. It is recommended to release 12.5 predatory mite/ m2 at weekly interval or 25 predatory mite/m2 on a biweekly interval mainly by hot spot treatments in highly infested areas.

On pepper plants, the population of spider mites was relatively low, the population did not exceed the threshold level. The highest ratio of spider mites: *Phytoseiulus* was 9.57:1 on May 29 and reduced to reach 0.56:1 on June 14. This shows that *P. persimilis* was more highly effective in the management of spider mite infestation on peppers. Aphids were present on peppers only, with a maximum average of aphid recorded of 0.53 aphid/ leaf. However, the aphid infestation was controlled by 3 sprays of *Beauveria* on hot spots only. The aphid population kept on fluctuating throughout the growing season. However, the natural introduction of *A. colemani* and *C. carnea*, kept the aphid population under control.

In conclusion, this study showed a high potential for the use of locally collected natural enemies to manage arthropod populations under normal greenhouse conditions. Based on these findings, entomopathogenic fungi can be cultured on different grains, with burghul and oats producing the highest yield of conidia at 1:1 ratio of water to grains. The addition of mannitol or 1% corn oil enhanced the germination of *Beauveria* conidia and thus may improve *Beauveria* efficacy against pests. Treatment with 10^8 conidia ml^{-1}+ 1% corn oil + 0.01% Tween-20 was effective in the control of aphids, whiteflies, and *T. absoluta* eggs. The high efficacy of the local strain of *P. persimilis* in the control of spider mites and its compatibility for combined treatment with *Beauveria* within an IPM program were proven in small scale trials and confirmed in commercial scale greenhouse trials.

Recommendations:

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

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

i. Expand the rearing production units, for sufficient production of natural enemies.

ii. Improve the mass production trials of *B. pseudobassiana* by applying the liquid fermentation procedure and blastospore production.

iii. Collect and evaluate other potential biological control agents for the control of various arthropod pests, i.e.: *Aphidoletes aphidimyza, Macrolophus pygmaeus.*

iv. Extend the research to cover other crops, mainly tomato, which is the major greenhouse crop in Lebanon.

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

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

ix. Improve greenhouse infrastructure and the installation of insect-proof nets prior to transplanting the crop to reduce arthropod pest migration into the greenhouse.

x. Recommend to the ministry of agriculture and to NGOs, to support production of natural enemies and to train farmers and extension officers on biologically based IPM.

The preliminary results of the biologically based IPM are very encouraging and it is highly recommended to intensify these studies for the safety of humans, wildlife and the environment. Lebanon is characterized by its biodiversity, where plenty of natural enemies could be collected and examined for their efficacy in pest management. Even though these alternative measures may look more expensive than pesticides, but they will reduce the pesticide residue levels and will allow the farmers to export their produce to the European Union or any other country, get higher prices for their produce and improve their income and wellbeing. The local production of natural enemies will reduce their cost and make their use more readily accepted by farmers.

APPENDIX I

BUFFERS

I. 2% CTAB Buffer

2% w/v CTAB

100mM Tris

20mM EDTA

1% w/v PVP

1.4M NaCl

Dissolved in 100ml autoclaved distilled water.

pH adjusted to 8 with HCl.

APPENDIX II

PESTICIDE SPRAYS

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

Natural enemy / Pesticides	*P. persimilis*
Acequinocyl	ST
Bifenazate	ST
Cyflumetofen	NT
Spirodiclofen	ST
Propamocarb-HCl	NT
Penconazole	NT
Kresoxim methyl	NT
Azoxystrobin	NT
Fenhexamid	ST
Myclobutanil	NT
Dimethomorph	NT
Fosethyl-aluminum	NT
Thiophanate-Methyl	T

Table II. Pesticide spraying program of the farmer throughout the growing period in the control greenhouse

eek	D ate	Nb of sprays	Active Ingredient	Trade Name
	27 /3/2019	3	Emamectin Benzoate	Super Apolone
			Acetamiprid	Asia
			Fenbutatin oxide	-
	3/ 4/2019	1	Mancozeb + Cymoxanyl	Zoral
			Acetamiprid	Optimal
			BT	Delfin
			Flonicamid	-
	10 /4/2019	1	Acetamiprid	Optimal
			Tolfenpyrad	Hatchi-Hatchi
			Mancozeb	Zoral
	17 /4/2019	0	-	-
	24 /4/ 2019	1	Chlorothalonil	Clortosip
	1/ 5/2019	2	Abamectin	SuperMect in
			Thiamethoxam	Flaxam
			Acetamiprid	Optimal
			Tolfenpyrad	Hatchi-Hatchi
			Nemacic	-
	8/ 5/2019	1	Thiamethoxam	Flaxam
			Acetamiprid	Optimal
	15 /5/2019	1	Mancozeb	Zoral
	22 /5/2019	1	Acetamiprid	Optimal
			Abamectin	SuperMect in
			Tolfenpyrad	Hatchi-Hatchi
			Carbosulfan	Marshal
	28 /5/2019	0	-	-
0	3/ 6/2019	2	Acetamiprid	Optimal
			Tolfenpyrad	Hatchi-Hatchi
			Thiamethoxam	Flaxam
			Mancozeb + Cymoxanyl	Zoral
	11	1	Abamectin	SuperMect

1	/6/2019			in
			Acetamiprid	Optimal
			Thiamethoxam	Insetto
2	14 /6/2019	0	-	-
2	18 /6/2019	1	Abamectin	SuperMect in
			Acetamiprid	Optimal
			Thiamethoxam	Flaxam
			Pyridapen	Pyrilux
4	21 /6/2019	0	-	-

Table III. Release and spraying program in the IPM greenhouse

eek	Date	Nb of sprays per week	Active Ingredient	Mode of Spray	Cro p
	27/3 /2019	2	Emamectin Benzoate	Gene ral	C+ P[1]
			Acetamiprid		
			Fenbutatin oxide		
		1	10000 Phytoseiulus		
	3/4/ 2019	1	200 Phytoseiulus	Hot spot	P
	10/4 /2019	1	10^8 Beauveria	Hot spot	P
	17/4 /2019	0	-	-	-
	24/4 / 2019	0	Chlorothalonil	Gene ral	C+ P
	1/5/ 2019	1	10^8 Beauveria	Hot spot	P
			Chlorothalonil	Gene ral	C+ P
	8/5/ 2019	1	700 Phytoseiulus	Gene ral	C
			10^8 Beauveria	Gene ral	P
	15/5 /2019	1	1000 Phytoseiulus	Gene ral	P
			Chlorothalonil	Gene ral	C+ P

	22/5/2019	1	1000 Phytoseiulus	General	C
			Chlorothalonil	General	C+P
	28/5/2019	1	5000 Phytoseiulus	General	C
0	3/6/2019	1	500 Phytoseiulus	Hot spot	C
1	11/6/2019	1	2000 Phytoseiulus	General	C
2	14/6/2019	1	9000 Phytoseiulus	General	C
3	18/6/2019	0	-	-	-
4	21/6/2019	0	-	-	-

1: C= Cucumber and P= Pepper crops.

APPENDIX III

TEMPERATURE AND RELATIVE HUMIDITY

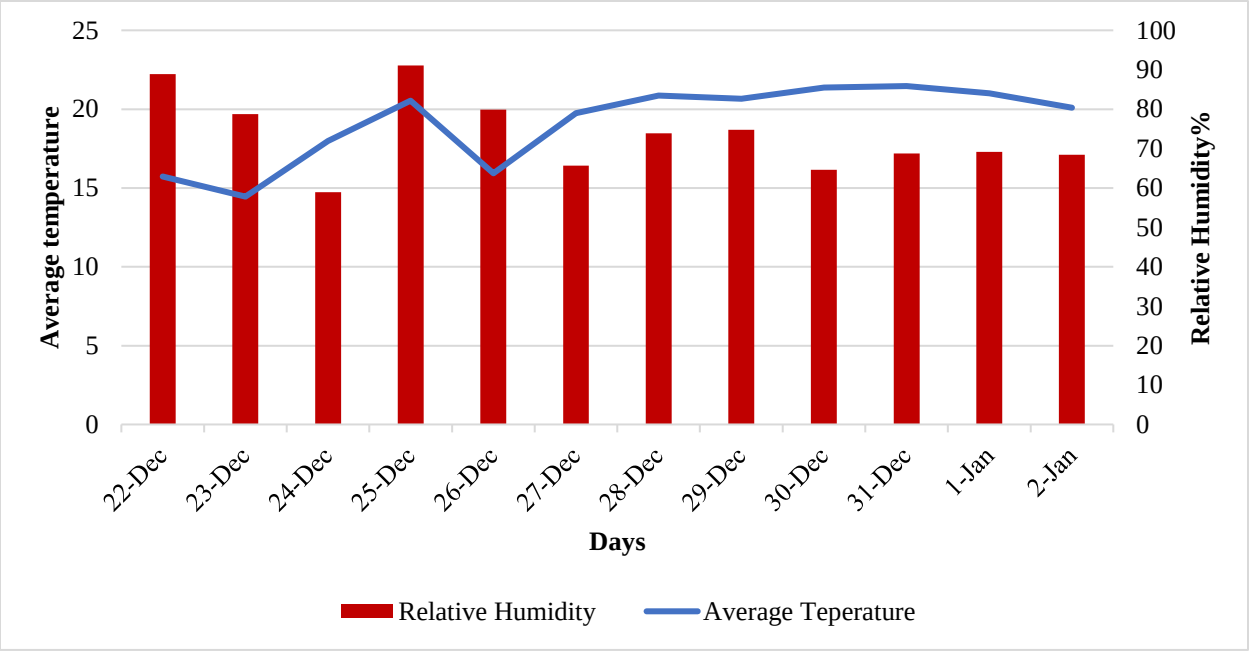

Figure I. Temperature and RH variations recorded throughout the cages experiment of *T. absoluta*

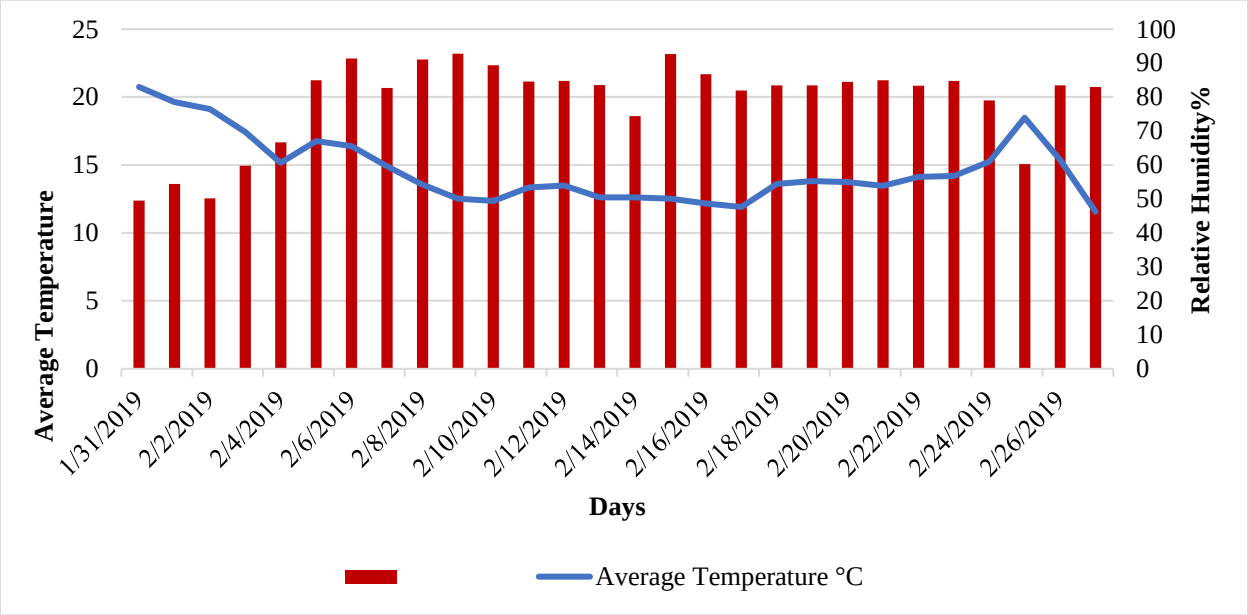

Figure II. Temperature and RH variations recorded throughout the greenhouse experiment of *T. absoluta*

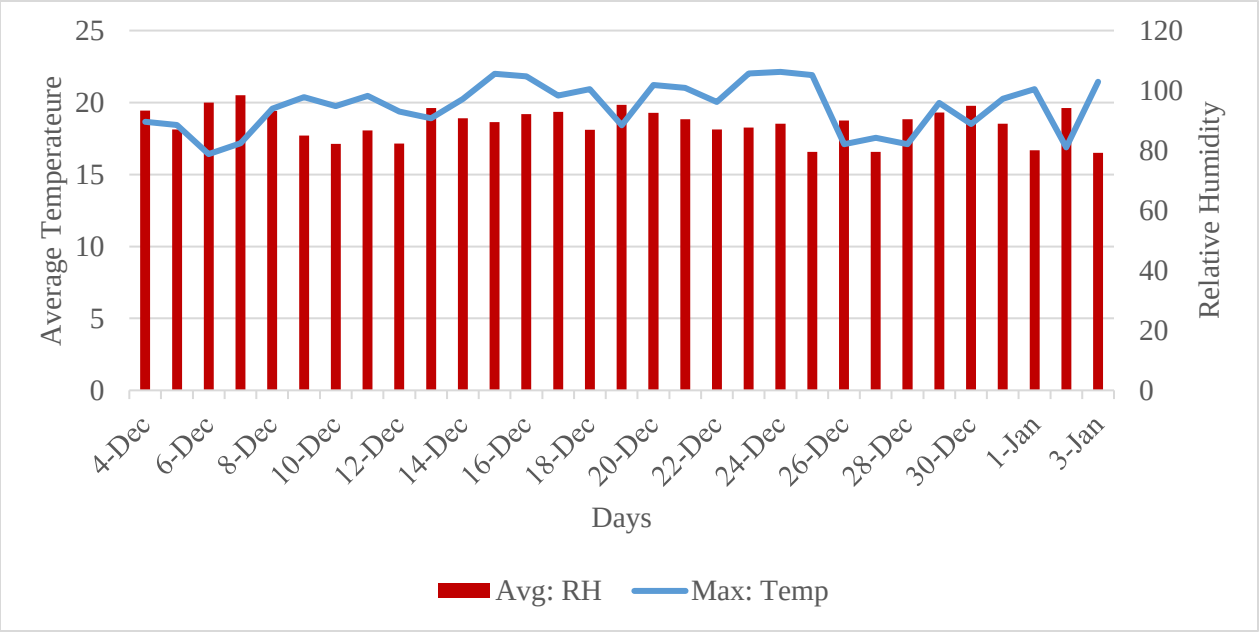

Figure III. Temperature and RH variations recorded throughout the preliminary experiment of Phytoseiulus in IPM

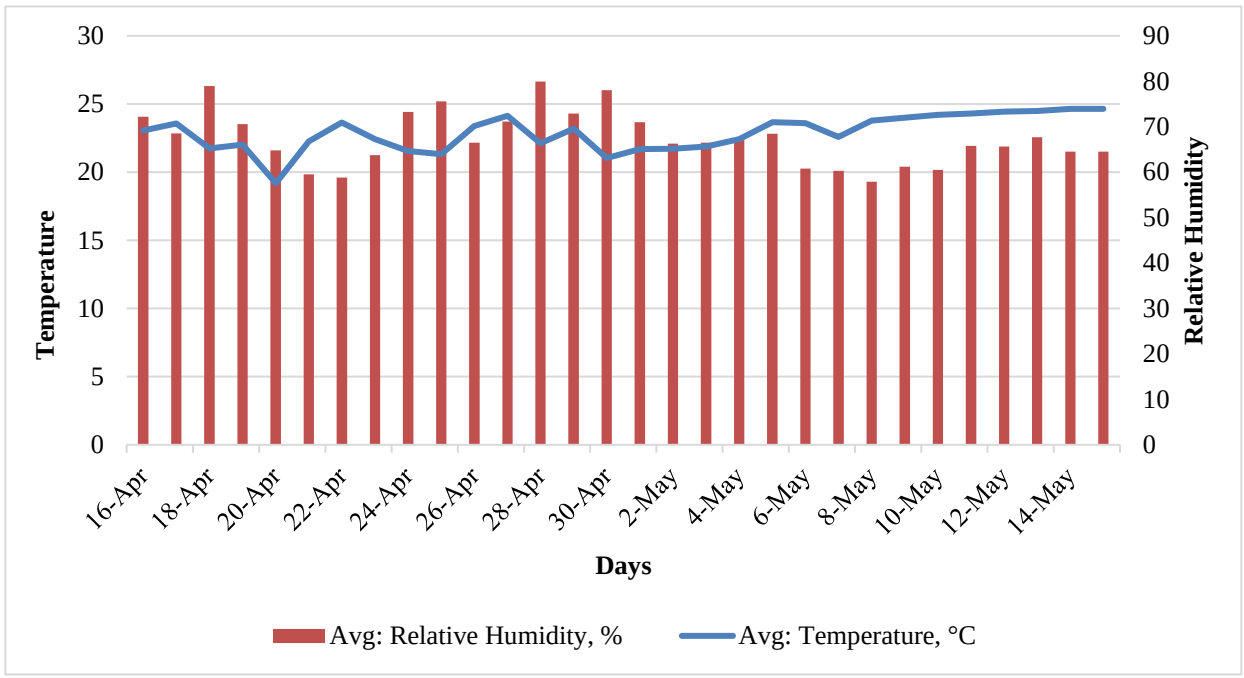

Figure IV. Temperature and RH variations recorded throughout the confirmation experiment of Phytoseiulus in IPM

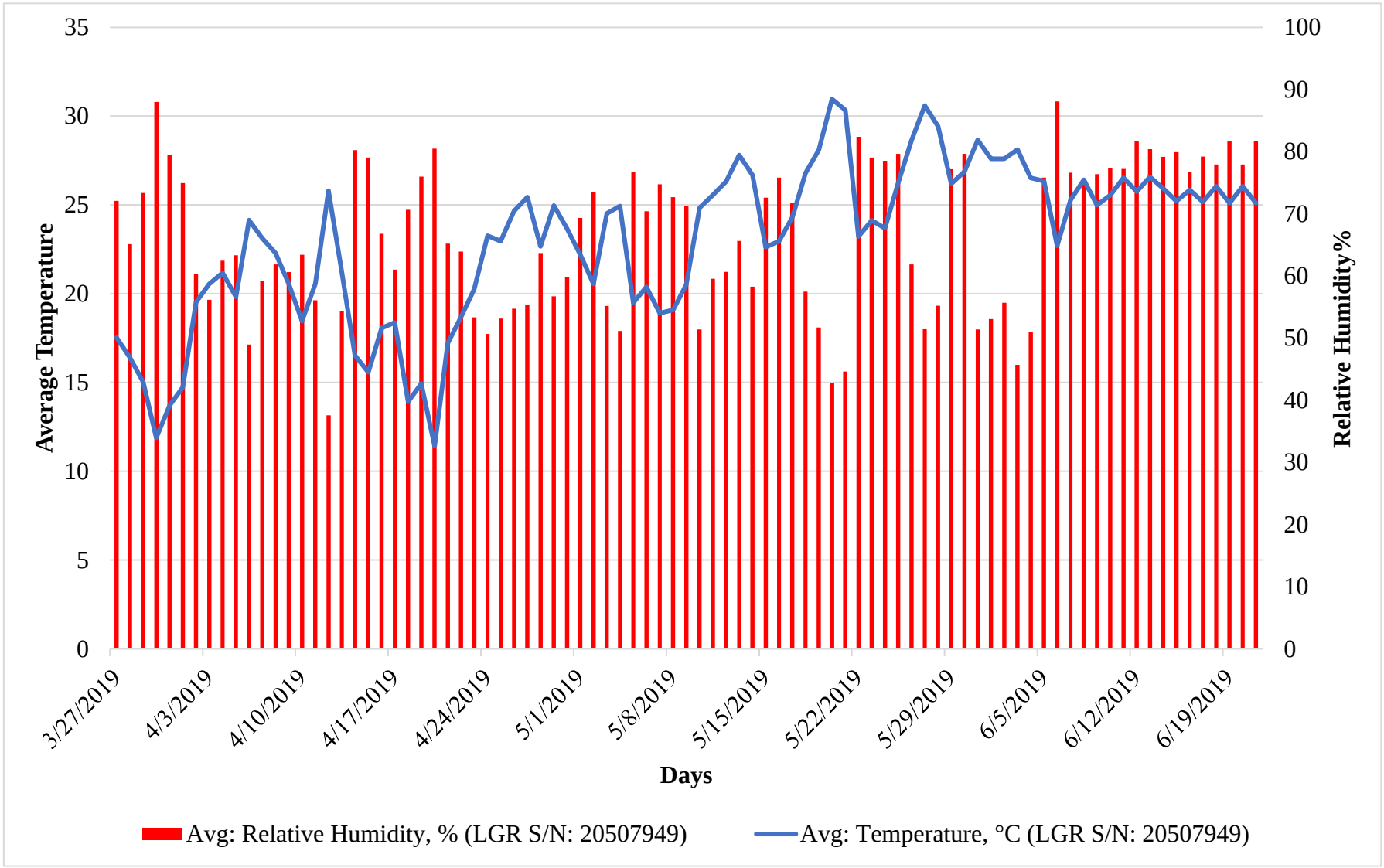

Figure V. Temperature and RH variations recorded throughout the experiment of Kfarmashoun

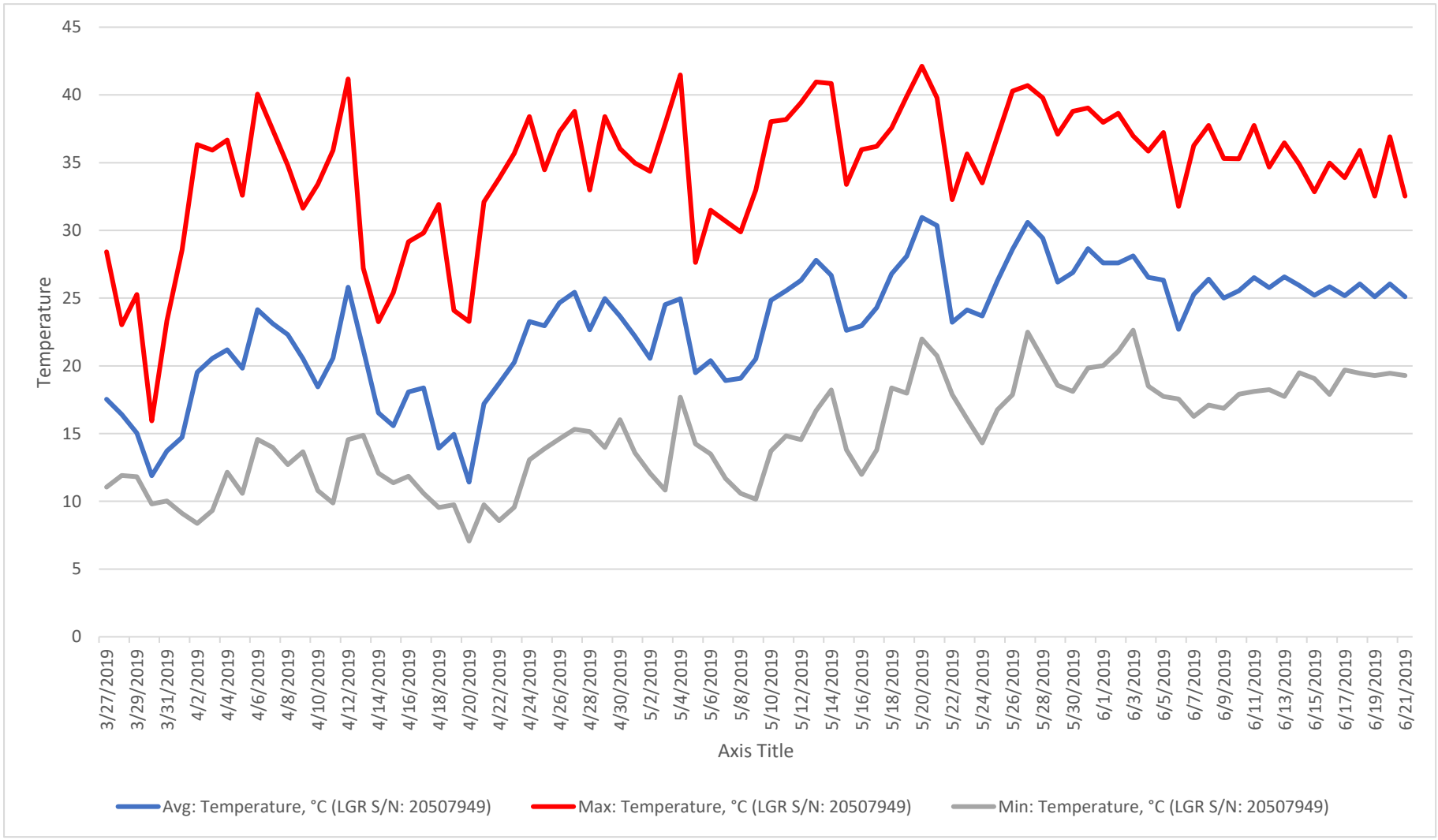

Figure VI. Maximum, minimum, and average temperatures throughout the experiment of Kfarmashoun.

APPENDIX IV

COST ANALYSIS

Table I. Estimated cost of imported biological control agents compared to the locally produced products, for a 450m^2 greenhouse.

Natural enemies	**Total numbers used per season in 450m^2**	**Cost per unit in country of origin**	**Cost per 450 m2 per season**	**Cost if local production 50%**	**Cost if local production 40%**	**Material cost for local production per unit**	**Cost per 450 m2 per season**
A.swirskii	107,000	207$	443$	221.5$	177.2$	Boxes 1$, Vaseline 0.5$ Yeast (20g) 0.2$ Wheat bran (100g) 0.1$ Wheat mite (0.9$) **Sum =1.8$**	14.5$
P. persimils	30,000	171$	513$	256.5$	205.2$	Seeds 12$, Pots 8$ Fertilizers &fungicides 5$ Vermiculite 0.5$, Net 3$ Bottle 0.5$ **Sum = 29$**	87$
B. pseudobassiana	10 L (10^8 conidia ml^{-1})	77$	2$	1$	1.2$	Bag 0.5$, Burghul 2$ Oil 0.27$, Tween 0.05$ **Sum=2.82$**	2.82$
Total cost			**958$**				**104.3$**

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