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SUSTAINABLE BIOCONTROL OF GREENHOUSE VEGETEABLE PESTS IN LEBANON: MASS PRODUCTION OF *AMBLYSEIUS SWIRSKII* AND ITS EFFICACY AGAINST WHITEFLIES AND THRIPS

by

FATIMA MAAN ABDALLAH

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FATIMA MAAN ABDALLAH

Approved by:

Dr. Yusuf Abou Jawdah, Professor — Advisor
Agriculture Department, FAFS

Dr. Nabil Nemer, Professor — Member of Committee
Agricultural Engineering Department, USEK

Dr. Mirella Aoun, Assistant Research Professor — Member of Committee
FAFS

Date of thesis defense: May 13, 2020

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AN ABSTRACT OF THE THESIS OF

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Title : Sustainable biocontrol of greenhouse vegeteable pests in Lebanon: Mass production of *Amblyseius swirskii* and its efficacy against whiteflies and thrips.

Pest attacks contribute to significant yield losses in vegetable production. Most farmers rely mainly on toxic synthetic pesticides to control these pests. However, due to the repeated use of pesticides and their misuse, pests, mainly whiteflies and thrips, have developed resistance to several pesticides; often leading to failure in pest control and/or a high number of pesticide applications resulting in great economic costs to farmers and negative impact on human and environmental health. Therefore, biological control can be an essential component in controlling these pests within an IPM strategy. *Amblyseius swirskii* (Anthias-Henriot), a beneficial predatory mite is used as biological control agent against a wide range of arthropod pests, including thrips, whiteflies, eriophyid mites, broad mites and spider mites. Since, at present, the Lebanese government doesn't allow farmers to import such arthropod natural enemies, our objective in this thesis was to search for these beneficial predatory mites in Lebanon, rear them, evaluate their efficacy in controlling the pests and hopefully at a later stage, supplying them to farmers. A local strain of *A. swirskii* was collected from Batroun area and was confirmed to be *A. swirkii*, using both morphological and molecular tools. Laboratory trials were performed to determine the best rearing media for *A. swirskii* and its prey, *Carpoglyphus lactis* (Linnaeus). Coarse autoclaved wheat bran along with 20% yeast was the best combination for rearing *C. lactis*, while 15 *A. swirskii* per gram of culture media of coarse autoclaved wheat bran along with 20% yeast with 1:50 *A. swirskii* to *C. lactis* ratio was successfully able to yield 300 *A. swriskii* per 1 g of culture media four weeks post inocculation. A small scale greenhouse trial on cucumber plants proved that the locally reared *A. swirskii* were capable of successfully controlling whitefly eggs with 90 and 97% and whitefly nymphs with 85 and 93 % efficacy, respectively at two release rates 50 and 100 *A. swirskii*/ m^2, five weeks post introduction. A large scale greenhouse experiment for evaluation of the efficacy of the local *A. swirskii* for the management whiteflies and thrips was carried in two commercial size greenhouses in Kfarmashoun.One greenhouse served as a control, where the farmer followed his normal plant protection measures while in the second one, IPM practices were implemetned.The populations of insects on cucumber and pepper leaves were monitored and recorded on weekly basis. With no insecticide sprays during the entire growing season, *A. swirskii* successfully maintained the thrips and whiteflies populations below their economic threshold level (ETL) with an efficacy similar to 14 insecticidal/acaricidal sprays in the control greenhouse. The maximum population of adult thrips, larvae thrips, whitefly adults and whitefly nymphs recorded were 1.54, 6.36, 1.65 and 1.45, respectively per cucmber leaf and 1.14, 0.4, 0.63 and 0.33, respectively per pepper leaf in the IPM greenhouse. These trials showed that the local *A. swirskii* could be mass reared in

laboratories under controlled environmental conditions, in order to be provided to Lebanese farmers to help them in reducing damage by the two major insect pests in cucumber and pepper greenhouses, with considerable reduction in insectiside sprays leading to minimizing risks to human health and the environment.

Keywords: *Amblyseius swirskii* , IPM, biological control, thrips , whiteflies

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ABBREVIATIONS

ai	Active ingredients
A. cucumeris	*Amblyseius (=Neoseiulus) cucumeris*
A. franciscana	*Artemia franciscana*
A. lycopersici:	*Aculops lycopersici*
A. pisum	*Acyrthosiphon pisum*
A. swirskii	*Amblyseius swirskii*
SW	*Amblyseius swirskii*
BT	*Bacillus thuringiensis*
BCA:	Biological control agent
BSC	Blue Sticky Card
C. lactis	*Carpoglyphus lactis*
CL	*C. lactis*
cm	Centimeter(s)
(C)	Coarse
°C	Degree Celsius
$	Dollars

ETL	Economic Threshold Level
E. kuehniella	Ephestia kuehniella
€:	Euro
EIP-AGRI:	European Innovation Partnership for *Agricultural* productivity and Sustainability
g	Gram(s)
ha	Hectare
IPM	Integrated Pest Management
ITS	Internal Transcribed Spacer
L	Liter(s)
M. caliginosus	*Macrolophus caliginosus*
µm	Micrometer(s)
mg	Milligram(s)
mL	Milliliter(s)
mm	Millimeter(s)
N. cucumeris	*Neoseiulus cucumeris*
O. laevigatus	*Orius laevigatus*
O. majusculus	*Orius majusculus*
/	Per

%	Percent of a hundred
P. persimilis	*Phytoseiulus persimilis*
P	Powder
RH	Relative Humidity
R. communis	*Ricinus communis*
m^2	Square meter
T. urticae	*Tetranychus urticae*
T. entomophagus	*Thyreophagus entomophagus*
T. casei	*Tyrophagus casei*
T. latifolia:	*Typha latifolia*
WB	Wheat Bran
WPI	Week post inoculation
YSC	Yellow Sticky Card

CHAPTER I

INTRODUCTION

Since the beginnings of agriculture, 10, 000 years ago, until present, growers have had to compete with harmful organisms including animal pests (insects, mites, nematodes, rodents, slugs, snails, and birds), plant pathogens (viruses, bacteria, fungi, and chromista) and weeds (i.e. competitive plants growing where they are not desired). These pests have been jeopardizing the productivity of crops that are cultivated for human use and consumption. Crops in different ecosystems have been under biotic and abiotic stresses which may be capable of reducing the sustainability of crop production. Abiotic stress is caused by the negative impact of non-living factors on the living organisms such as temperature, sunlight, wind, salinity, flooding, drought… In contrast, biotic stress is due to the damage of a living organism caused by another living organism such as insects, fungi, bacteria, viruses, … According to National Institute of Food and Agriculture (NIFA), 40-50 percent of the crop yield in the developing countries are lost due to pests, or post-harvest losses. Conventional crop protection strategies relied mainly on chemical pesticide application, often preventive, or calendar applications of broad-spectrum fungicides, insecticides, acaricides, nematicides, molluscicides and herbicides (Horne & Page 2008). Crop protection measures include several other management strategies that prevent or reduce these harmful organisms and thus reduce crop losses including applying physical (cultivation, mechanical weeding, netting etc.), cultural (cultivar choice, crop rotation,

antagonists, predators, etc.) and/or chemical treatments (pesticides). The overuse of pesticides resulted in the building up of resistance by pests to the active ingredients and pathogens (Helyer, 2014). Furthermore, with the growing public awareness concerning pesticide residues, regulations are being set by the European Union (EU) to limit the use of broad-spectrum chemical pesticides and to shift to selective or biorational pesticides. Therefore, biological control agents (BCAs) are gaining importance as they are competing with pesticides in controlling pests and pathogens in high value vegetable and ornamental crops (Lenteren,2012).

The scope of the present research concentrates on:

1. Molecular identification of some local *Amblyseius swirskii* specimens
2. Evaluation of different media for mass rearing *Amblyseius swirskii* and *Carpoglyphus lactis*
3. Evaluation of the efficacy of the local *A swirskii* for management of two major greenhouse vegetable insect pests: whiteflies and thrips

CHAPTER II

LITERATURE REVIEW

A. Overview of protected cultivation

Controlled environment or greenhouse food production is nothing new. In fact, it dates back to Roman times where gardeners used to grow cucumber in artificial methods because the Doctors prescribed a cucumber a day for the Roman emperor Tiberius (Shamshiri, 2007).

Commercial protected horticulture appeared first in Northern Europe in the early decades of the last century and developed broadly after World War II. In mid-20th century, protected cultivation was introduced to the Mediterranean regions (Pardossi et al., 2004). According to the EIP-AGRI, the area under protected cultivation is steadily increasing in the EU. In 2015 the estimated total area in the EU was about 175,000 hectares (ha), with a rate of increase of approximately 4.5% between 2005 and 2013. In the Mediterranean region, protected cultivation constitutes the most productive form of primary agricultural production; a total area of about 120,000 ha was recorded in 2016. Greenhouse cultivation can provide high-quality products all-year round with an efficient use of resources, such as water, fertilizers, pesticides and hand labour (Pardossi et al., 2004). The main crops cultivated are vegetables with tomato and cucumber covering almost 70% of the cultivated area (EIP-AGRI, 2019).

B. Cucumber production

Cucurbits are the popular name of the family Cucurbitaceae which is represented by 130 genera and 800 species with about 90 genera and 700 species mainly used for food as they are among the most economically important vegetable crops worldwide (Weng and Zhanyong, 2012).Cucurbits are widely distributed in the tropics and warm temperate region (Rahman et al., 2006). Major cucurbit crops include cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], and squash or pumpkin (*Cucurbita pepo* L., *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch.) (Weng and Zhanyong, 2012). Cucumber is one of the most popular greenhouse vegetable products worldwide. It is a warm-season plant and grows rapidly at 24–29 °C. China is the world's largest producer of cucumber and gherkins with the production of 56,293,530 tons during 2018 followed by Iran and Turkey with 2,283,750 and 1,848,273 respectively (FAOSTAT, 2019). In 2016, Lebanon produced 177,831 tons of cucumber and gherkins ranking it at place 25th among the world leaders in cucumber production worldwide (Naegele and Wehner, 2016). With a total production of 151,558 tons, cucumber ranked third in the top ten commodities produced in Lebanon in 2017. Potato production ranked first followed by tomato production with 387,791 tons and 300,157 tons, respectively, in 2018 (FAOSTAT, 2019).

C. Pepper production

The Solanaceae family is made up of 3000–4000 species that are classified in approximately 90 genera. The most important vegetable genera are *Solanum* (potato and eggplant), *Lycopersicon* (tomato), and *Capsicum* (pepper) (Gebhardt, 2016). Peppers are

warm-season crops, sensitive to freezing temperatures at any growth stage (Gebhardt, 2016).The world production of chilies and pepper was 36.7 million tones cultivated on 1.99 million ha during 2018, with China ranking as the top 1 producer with 16.1 million tons followed by Mexico and Turkey with 2.48 and 2.15 million tons, respectively. Pepper is among the major vegetable crops in Lebanon with a total production of 23,862 tons on cultivated area of 604 ha during 2018 (FAOSTAT, 2019).

D. Major pests on cucumber and pepper

1. Thrips

Thrips are very small, cosmopolitan insects, measuring between 0.1-0.15mm in length. There are around 6,000 thrips species worldwide, but only 1% of these species are considered as agriculturally important pests and most of these belong to the family Thripidae (Turcios et al.,2015). *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Thrips palmi* (*Karny*) (Thysanoptera : Thripidae), and *Thrips tabaci* (Lindeman) (Thysanoptera:Thripidae) are the main agricultural pests; as they cause direct and indirect damage mainly to cucumber, pepper, tomato, onion and zucchini (Turcios et al.,2015). Direct damage is caused by the feeding habit of the adults and nymphs on plant cell contents and thus decreasing the ability of the plant to undergo photosynthesis. Indirect damage is due to the transmission of plant viruses such as "*Tomato spotted wilt virus*" (TSWV) and "*Impatiens necrotic spot virus*" (INSV) (Shipp et. al,2000). The eggs of thrips are laid inside the plant tissue. After hatching, two actively feeding larval instars start feeding on the plant tissue. At the end of second larval stage, thrips stop feeding as they fall down to the ground where they pupate in the soil or on the lower leaf surfaces. Then non-

feeding quiescent pre-pupal and pupal stages are formed before reaching the adult stage (Lee, 2017). The optimum development of thrips is at 30^{o}C; decline in its development is observed at temperatures below 10^{o}C or above 35^{o}C (Malais,2004). Farmers mainly used chemical control measures to control thrips such as organochlorines, organophosphates, pyrethroids, carbamates, spinosyns and avermectins (Kliot et al., 2016). However, the overuse of these pesticides helped in building up of resistance to these chemicals. For example, during 1961 *F. occidentalis de*veloped resistance to the pesticide toxaphene on cotton. Thus, alternative control measures should be adopted to maintain the economic injury levels (EILs) for *F. occidentalis* below 20 to 50 adults per sticky trap per day, 3 to 7.5 adults per flower , 1.7 adults or 9.5 larvae of *F. occidentalis* per middle leaf of greenhouse cucumber (Shipp et. al, 2000).

2. Whiteflies

The whitefly, *Bemesia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important agricultural pest of vegetables, ornamental and field crops worldwide (Vassiliou et al. ,2011). Whiteflies have six life stages: the egg, four nymphal instars, and the adult stage (Perring et al.,2018). Out of many known *B. tabaci* strains, the most common ones in the Mediterranean regions are Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) species (De Barro et al., 2011). Three types of damages are caused by the whiteflies: direct damage, indirect damage and virus transmission (Baroudy, 2010). Direct damages are due to the piercing and sucking of the plant foliage which lead to early wilting of the plant, reduction in growth rate and yield. Indirect damage through the accumulation of honeydew which serves as a substrate for the growth of black sooty mold on leaves and fruit resulting

in reduced photosynthesis (Baroudy,2010). The third and main concern about whiteflies is that they are capable of transmitting several plant viruses. Out of 1,500 whitefly species, only few are vectors of plant viruses, and these vectors include the cotton/tobacco/sweet potato whitefly (*Bemesia tabaci*) and the greenhouse whitefly (*Trialeurodes vaporariorum)* (Westwood) (Navas-Castillo et al., 2011).

The main control strategy adopted by farmers to control whiteflies was limited to the use of chemical insecticides. However, in many vegetable cropping systems, intensive use of insecticides was required to achieve an effective control of *B. tabaci*, which lead to the development of insect resistance to organophosphates, pyrethroids, carbamates and chlorinated hydrocarbons (Nauen & Denholm, 2005). Therefore, farmers started using newer compounds like insect growth regulators (IGRs) and neonicotinoids as they were considered effective in controlling *B. tabaci.* However, the effectiveness of these new compounds didn't last long, since starting 1996, evidence of *B. tabaci* resistance to imidacloprid (neonicotinoid) was reported (Elbert & Nauen, 2000). Later, evidence of biotypes MEAM1 and MED resistance to several neonicotinoids including thiamethoxam were reported. (Kliot et al., 2016). Thus, an alternative control strategy should be adopted to maintain *B. tabaci* and *T. vaporariorum* on cucumber plant below their ETLs (4.6 and 5 whitefly adults per leaf, respectively) (Jeon et al., 2009).

3. Spider mites

Out of the known spider mite families feeding on higher plants, Tetranychidae, Tarsonemidae, and Eriophyidae pose the greatest threat to agricultural crops (Malais & Ravensberg, 2004). *Tetranychus urticae* (Koch) (Acari: Tetranychidae) is a major pest of

many crops, including cotton, ornamentals, strawberries, watermelons, tomatoes and others (Sato et al., 2005) . Mites belonging to the Tetranychidae family have five developmental life stages; egg, larval stage, two nymphal stages and adult stage. *T. urticae* overwinters as adults in the soil or on weed hosts. It is soft bodied, oval-shaped, the back arched and bearing bristles measuring about 0.3 to 1 mm in length and has two dark spots (sometimes four), one on either side of the top. The color can vary from greenish or yellowish, pearly amber, to red depending upon the host plant and environment.

Developmental rate is significantly determined by temperature. Under greenhouse conditions, the average development time from egg to adult is 14-21 days. However, mites develop quickly under hot, dry conditions and may mature in as few as 7 days during these periods. But development stops at temperatures below 12^{o} C and is retarded at temperatures above 40^{o} C (Malais & Ravensberg, 2004). Eggs are laid singly on the surface of leaves. The eggs are spherical and found on the underside of the leaf often where mite feeding is noticeable (Malais & Ravensberg, 2004). All active developmental stages of the two-spotted spider mites damage the host through feeding on the underside of leaves and sucking its sap. Thus, the photosynthetic potential of the host decreases and unfavorable symptoms such as spotting and webbing develop on leaves and fruits, leading to unmarketable products (Malais & Ravensberg, 2004). Its phytophagous nature, high reproductive potential and short life cycle facilitate rapid resistance development to many acaricides often after a few applications (Sato et al., 2005). Sato et al. (2016) reported spiromesifen resistance in *T. urticae* on several crops in Brazil. Moreover, evidence of resistance to bifenthrin, abamectin and hexythiazox was reported in Turkey (İnak et al.,

2019). Thus, resistance management strategies should be adopted and implemented by farmers to maintain the economic threshold below 5 mites per leaf (English-Loeb, 2003).

4. Aphids

Aphids (Hemiptera: Aphididae) are herbivorous insects accounting to more than 4,300 described aphid species, but only around 100 have successfully exploited the agricultural environment to become economically important pests (Silva et al., 2012). The most important greenhouse aphid species are the green peach aphid (Myzus *persicae)* (Sulzer), cotton aphid (*Aphis gossypii)* (Glover) and potato aphid (*Macrosiphum euphorbiae*) (Thomas). Most aphid species have an annual holocyclic life cycle, which consists of one generation of sexual individuals, followed by several parthenogenetic generations. About 90% of the species are monoecious species where they complete their life cycle on the same host plant and the remaining 10% are heteroecious species whereby they alternate between a primary and a secondary host (Arounet al., 2015). They damage the crop by direct feeding through inserting their stylet into the plant leaves to suck the plant sap and thus reducing the yield, but their primary importance as pests is their ability to transmit plant viruses (Griffin and Williamson,2019). They transmit *Lettuce necrotic yellows virus* (*Rhabdoviridae)* and *Cucurbit aphid-borne yellows virus (Luteoviridae)* in a circulative propagative manner, *Barley yellow dwarf virus (Luteoviridae)* and *Potato leaf roll virus* (*Luteoviridae*) *virus* in circulative non propagative manner, *Cauliflower mosaic virus* (*Caulimoviridae*) and *Beet yellows virus (Closteroviridae)* in noncirculative, semipersistent manner and *Cucumber mosaic virus (Bromoviridae)* and *Potato virus Y* (*Potyviridae)* in a noncirculative, nonpersistent manner. A circulative virus is when the virus crosses the gut

barrier and enters the circulatory system of the insect and accumulates inside the salivary glands. They are further classified as circulative non-propagative or circulative propagative, depending on whether the acquired virus replicates within the vector. However, non-circulative viruses have a more superficial and transient relationship with the vector where the virus remains attached to the cuticle of the insect mouthpart of foregut and do not cross the gut barrier (NG et al.,2004; Kassem et al., 2007).

Growers relied exclusively on chemical insecticides to control *M. persicae* thus leading to development of multiple forms of insecticide resistance. The first evidence of *M. persicae* resistance was for organophosphates reported in 1955, while now resistance has been reported to most classes of insecticides, including organophosphates, carbamates, pyrethroids, cyclodienes, and neonicotinoids making *M. persicae* one of the most widely and strongly resistant species worldwide (Bass,2014). An alternative control strategy should be adopted to maintain *M. persicae* below the economic threshold level of 20 aphids per Chinese plant (Jeon et al, ,2008).

E. IPM

The use of pesticides made increase crop yield possible by reliance on simple cropping systems. However, the over reliance on these chemicals threatened not only the ecosystem, but also the human health. It also led to the emergence of pest resistance and declining availability of active substances. The European Crop Protection Association stated in their study that there were 70 new active ingredients during 2000, while during 2012 the number was 28 new ai only (McDougall ,2013). Resistance to pesticides was the main driving force which made growers shift from chemical to non-chemical control measures.

Using BCAs only is not enough for controlling pests and diseases that crops are exposed to. They are usually used along with biorational chemicals in integrated pest management (IPM) programs. According to The European Union , IPM is defined as the “careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep plant protection products and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms (Directive 2009/128/EC) (Barzman et al., 2015). In other words, IPM is an ecosystem-based strategy that focuses on long-term prevention of pests or their damage through a combination of techniques including preventing and suppressing pests; scouting/monitoring of harmful organisms; relying on cultural, physical, mechanical and biological control measures in preference to chemical measures; application of target-specific pesticides; limiting the use of synthetic chemicals; implementing anti-resistant strategies like applying pesticides having different modes of action; and record keeping (Barzman et al., 2015). On the other hand, IPM is a system where the effort to control pest, emphasizes on non-chemical methods and chemicals are only applied when there are no alternatives. The approach is viable in all three important dimensions of sustainability, such as social, economic and environmental.

F. Biological control

Biological control (biocontrol) is defined as the use of one living organism to reduce the population density of another organism, making it less abundant and less damaging (van

Lenteren, 2012). It has been in use for at least 2000 years, but modern use started at the end of the nineteenth century (van Lenteren et al., 2018). It consists of the beneficial action of parasites, pathogens, and predators in managing pests and their damage (Eilenberg et al., 2001).

1. *Different types of biological control*

a. Parasites and Parasitoids

A parasite is an organism that forms a nonmutual relationship with organisms of different species whereby the parasite benefits from the host for its development and survival (Helyer et al., 2014). Parasitoids usually have a free-living adult stage and a larval stage that develops on or within a single host organism, ultimately killing the host, which is a great benefit in terms of biological control. Parasitoids can be further classed as ectoparasitoids (ie: *Eretmocerus eremicus*) that live and attack from the outside of the host and endoparasitoids (ie: *Encarsia formosa*) that develop from an egg laid within the host (Helyer et al., 2014).

b. Pathogens

Natural enemy pathogens are microorganisms including certain bacteria, fungi, nematodes, protozoa, and viruses that can infect and kill the host. They kill by parasitism either directly or as a result of toxins that destroy the hosts' internal organs, allowing the pathogen to reproduce.

Some beneficial entomopathogens include *Bacillus thuringiensis or Bt*, entomopathogenic nematodes, and granulosis viruses (Helyer et al ,2014).

c. Predators

Predators kill and feed on several individual preys during their lifetimes. Many species of amphibians, birds, mammals, and reptiles prey extensively on insects. Predatory beetles, flies, lacewings, true bugs (Order Hemiptera), and wasps feed on various pest insects or mites. Most spiders feed entirely on insects. Predatory mites that feed primarily on pest spider mites include *Amblyseius* spp., *Neoseiulus* spp., and the western predatory mite, *Galendromus occidentalis* (Helyer et al., 2014).

2. *Methods of biological control*

There are four strategies for the use of biological control as mentioned by Helyer et.al (2014)

a. Conservation/Preservation Biocontrol

This method is based on the modification of the environment to enhance the establishment and survival of naturally occurring beneficials. It is mainly used in open fields and includes hedgerows, banks and flower margins providing alternative habitats for natural enemies along cultivated fields (van Lenteren, 2012).

b. Importation/Classical Biocontrol

This method is based on the introduction of an exotic organism for permanent control of an invasive non-native species of the pest. Beneficials are imported from the area where the pest originates to be released in the area where the pest was accidently introduced.

c. Inoculation biocontrol

It involves releasing small numbers of natural enemies throughout the pest period, starting when the pest population is very low. The natural enemies are expected to reproduce successive generations for prolonged, but not permanent, control. The expected outcome of inoculative releases is to keep the pest at low numbers, never allowing it to approach an economic injury level; therefore, it is more of a *preventive* measure.

d. Inundation biocontrol

Inundation involves mass release (large numbers) of natural enemies for immediate pest control. It is not dependent on successive generations of the biocontrol agent; therefore, it is more of a *corrective* measure.

In commercial biocontrol, preventive (inoculative) and corrective (inundative) releases are integrated and the term augmentative biocontrol is used to cover both types (Gerson et al, 2003).

The goal of biocontrol is to suppress the pest population and maintain it below the economic damage threshold of the crop and not to eradicate the pest; therefore, it doesn't control the pest and disease 100% (Lazarovits et al. 2007). Until 2018, BCAs were applied on more than 30 million ha worldwide with Europe being the largest commercial market for invertebrate biological control agents and North America has the largest sales of microbials (van Lenteren, 2018). There are 30 species of the biocontrol agents which make up for 90 % of the market value (Cock et al., 2010). The global market for BCAs is estimated to be US$1.7 billion in 2015 while the global pesticide market had a value of US$ 58.46 billion

(van Lenteren, 2018). With the shift from chemical control to biocontrol mainly due to insecticide resistance, the biocontrol market has increased significantly especially in Europe toward the end of the 20th century (Cock et al., 2010). Several BCAs which are available in the market are compatible with each other and can be used together to control the same or different pests.

More than 230 species of invertebrate natural enemies were used for pest management worldwide, 170 species used in augmentative biocontrol in Europe (Cock et al 2010) as they all originated from ten taxonomic groups. Out of the 230 (95.2%) species, 219 belong to the Arthropoda, 10 species belong to the Nematoda and one belongs to Mollusca. Within the arthropods, four taxonomic groups provided most natural enemies: first of all, the Hymenoptera (52.2%, 120 species), next the Acari (13.1%, 30 species), followed by the Coleoptera (12.2%, 28 species) and Heteroptera (8.3%, 19 species) (Cock et al 2010). However, collection of new data in 2016 showed the use of almost 350 natural enemies' species (van Lenteren, 2018). The first BCAs used in augmentative biocontrol were *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae) and *Chilocorus circumdatus* Gyllenhal (Coleoptera: Coccinellidae) starting 1902 (van Lenteren, 2012). Acarid predators are popular among the biocontrol agents because they can be mass reared easily, released by mechanical means, may control several pest species and are target specific because they do not spread actively over large distances and are relatively small. An example of a recent Acarid species becoming very popular in use is *Amblyseius swirskii* (Calvo et al., 2011). It is used in combination with biorational chemicals in integrated pest management programs.

G. Phytoseiid mites

The Phytoseiidae is one of the largest families of the mites (Acari) of the suborder Mesostigmata. Phytoseiidae are a cosmopolitan family, with almost 1800 species described worldwide, including specialist monophagous predators to generalist polyphagous predators (de Moraes,2004). They are of great economic importance for both growers and the biological control industry since they are efficient predators of phytophagous mites, thrips and whiteflies (Gerson et al.,2003). 25 species of the phytoseiid mites are sold commercially for biocontrol with three of which, *A. swirskii*, *N. cucumeris* and *P. persimilis*, ranking in the most important top ten BCAs (van Lenteren, 2012).

1. *Biology of phytoseiid mites*

The life cycle of phytoseiid mites consists of five developmental stages: egg, larva, protonymph, deutonymph and reproductive adult with relatively short development time (Fouly et al. ,2011). According to Chant and McMurtry (2007), phytoseiid mites have fewer than 23 pairs of setae. The male's spermatodactyl is located on the chelicerae and the females have a pair of spermatheca between coxae III and IV. For sensing their surrounding and prey, they rely on the mechano- and chemoreceptors on their abdomen, legs and at particularly high concentration in the anterior pair of legs and not on "eyes" which are not present.

Phytoseiids feed on their prey by piercing through the integument of their mobile prey or the egg chorion using the chelicera in order to consume the liquid contents inside (Gerson et al. 2003). Mated females are the most effective predators requiring

biomass for egg production, whereas males are comparatively ineffective. When food is abundant phytoseiids have an efficient egg production converting up to 70% of the consumed biomass into eggs (Nguyen, 2015).

In a healthy population the sex ratio is approximately 0.67, i.e. 2 females per male, but this can shift under stress such as overcrowding, dehydration, prey type or low food availability (Gerson et al. 2003). In highly female biased populations, maximum oviposition rate may not be achieved due to infrequent mating (Swirski et al. 1967). Mating occurs by venter- to-venter position with the smaller male hanging underneath the female with the spermadactyls inserted into the spermatheca (Nguyen, 2015). Females may emit a sex pheromone that helps the males locate a potential mate (Hoy & Smilanick 1979). Mating can be a long process, with the duration of copulation for *A. swirskii* recorded to be between 210-270 min (Nguyen, 2015). The population growth is not only based on the fecundity rate only, but it is also affected by the egg hatching success, juvenile survival and development time. High relative humidity (RH), 70-90%, is required by the eggs in order to avoid egg desiccation (Ferrero et al., 2010).

2. *Lifecycle of phytoseiid mites*

McMurty and Croft (1997) categorized phytoseiid mites into four different types according to their feeding behavior (Fathipour and Maleknia, 2016).

Type I lifestyle—**Specialized mite predators**

Subtype I-a—Specialized predators of *Tetranychus* (Tetranychidae)

Subtype I-b—Specialized predators of web nest-producing mites (Tetranychidae)

Subtype I-c—Specialized predators of tydeoids (Tydeoidea)

Type II lifestyle—**Selective predators of tetranychid mites**

Type III lifestyle—**Generalist predators**

Subtype III-a—Generalist predators living on pubescent leaves

Subtype III-b—Generalist predators living on glabrous leaves

Subtype III-c—Generalist predators living in confined space on dicotyledonous plants

Subtype III-d—Generalist predators living in confined spaces on monocotyledonous plants

Subtype III-e—Generalist predators from soil/litter habitats

Type IV lifestyle—**Pollen feeding generalist predators**

Type III is the most numerous type. It has an advantage where it targets several pests and non pest food source which allows prolonged persistence in the crop.

H. Amblyseius swirskii

1. *Classification*

KINGDOM Animalia

SUBKINGDOM Eumetazoa

PHYLUM Arthropoda

SUBPHYLUM Chelicerata

CLASS Arachnida

SUBCLASS Micrura

INFRACLASS Acari

SUPERORDER Anactinotrichida

ORDER Mesostigmata

SUBORDER Dermanyssina

SUPERFAMILY Ascoidea

FAMILY Phytoseiidae

GENUS *Amblyseius*

SPECIES *swirskii* Athias-Henriot

(Nguyen, 2015)

2. *Distribution*

A. swirskii was first reported in Beit Dagan, Israel in 1962 on *Prunus amygdalus* by Anthias-Henriot (1962). It is native to the Eastern Mediterranean region. It occurs naturally in Israel, Italy, Cyprus and Egypt on various crops including apples, apricot, citrus, cotton and vegetables (Doğramaci et al.,2013). In 1983, it was first released in North America to control citrus pests in California. Later starting 2005, *A. swirskii* has been released in several countries including Austria, Belarus, Belgium, Denmark, Finland, France, Germany, Greece, Guernsey, Hungary, Italy, Jersey, Morocco, the Netherlands, Norway,

Poland, Spain, Turkey, and UK in Europe, Argentina and Brazil in South America , Saudi Arabia, China ,Japan and several other countries in North America and North Africa (Nguyen, 2015).

3. *Morphology and identification*

(Nguyen, 2015)

a. Eggs

The eggs of *A. swirkii* are pale-whitish oval shaped with 0.20 mm in length and 0.15 in width (Figure 1). Eggs are laid on the underside of plant leaves at the intersection of the main and lateral rib of leaf hairs (trichomes) near plant domatia, an adaptation mechanism to avoid egg predators.

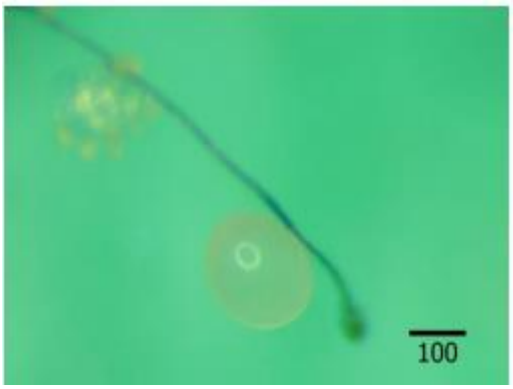


Figure 1. Egg (right) and empty eggshell (left) of *Amblyseius swirskii*. Scale in μm (Nguyen, 2015)

b. Larvae and nymphs

The larvae of *A. swirskii* are pale-whitish to transparent in color with 0.22mm in length and 0.16mm in width. The larva has three pairs of legs only (Figure 2).

Figure 2. Larva of *Amblyseius swirskii* with three pairs of legs. Scale in μm (Nguyen, 2015).

The protonymph (2nd stages) and deutonymph (3rd stage) are 0.26 mm in length, 0.16 mm in width and 0.28-0.34 mm in length, 0.16-0.19 mm in width, respectively, where differences in sizes between males and females are noticeable. Both the protonymph and deutonymphs have four pairs of legs and are darker in color compared to the larvae (Figure 3).

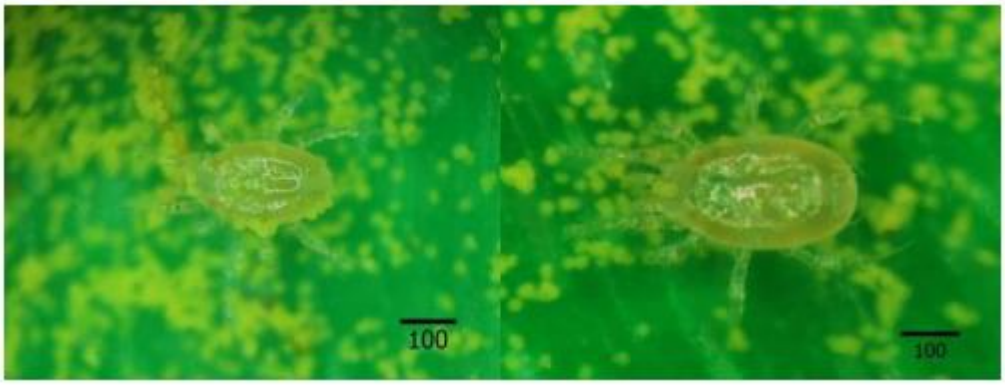


Figure 3. Protonymph (left) and deutonymph (right) of *Amblyseius swirskii*. Scale in μm (Nguyen,2015)

c. Adults

Adult females of *A. swirskii* are shiny, pear shaped with 0.5mm in length and 0.3 mm in width with four pairs of legs. However, males *A. swirskii* are oval shaped as they are smaller than the females with a length of 0.3 mm and 0.17 mm width (Figure 4). The males

are faster than the females and nymphs as they mate with the females directly after molting. The color of the adult *A. swirskii* varies depending on its diet. Adults that feed on thrips and whiteflies are pale yellow to pale tan however, the ones feeding of eggs of aphidophagous gall midge *Aphidoletes aphidimyza* are yellowish-brown to red in color.

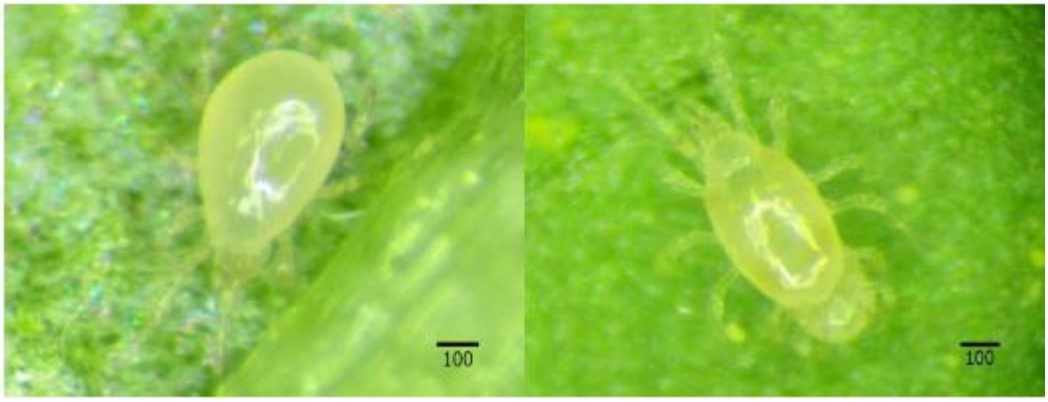


Figure 4. Female adult (left) and mating adult pair (right) of *Amblyseius swirskii* (Nguyen, 2015)

4. *Bioecology*

a. Development

The life cycle of *A. swirskii* consists of five developmental stages: egg, larva, protonymph, deutonymph and adult. The environmental conditions and the type of food consumed by the predatory mite influence its rate of development. For example, immature developmental time of *A. swirskii* varies from 4.8 days when it feeds on *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) at 26°C to 24.1 days when it feeds on *Zea mays* L. at 15°C (Allen, 2010). The development time of *A. swirskii* feeding on a living prey is faster than the one feeding on pollen where mite reared on *Aculops lycopersici* (Massee) (Acari:Eriophyidae) or *Tetranychus urticae* Koch (Acarina: Tetranychidae) was shorter than on pollen of *Typha latifolia* L. or *Ricinus communis* (L.) (Park et al., 2011).

Environmental conditions including temperature and relative humidity are also critical for the development of *A. swirskii* where the optimum temperature for its development is 25°C with 70-85% RH. The development time of the predatory mite decreases as the temperature increases from 15°C to 35 °C. No egg hatching at temperature 13ºC and 60% RH, while at temperatures exceeding 30ºC at 70-75% RH or 32ºC at 60% RH, the development of *A. swirskii* slowed down (Xia et al., 2011). *A. swirskii* is not susceptible to diapause (Bolckmans et al., 2005) it can be used throughout much of the season provided daytime temperatures regularly exceed 22°C (Bolckmans et al.,2005).

b. Reproduction:

Similar to other phytoseiid species, female *A. swirskii* should mate in order to produce eggs. Several factors including the food consumed, food quantity and the environmental conditions affect the reproduction rate of *A. swirskii*. First, mites feeding on a living prey such as *A. lycopersici* or *T. urticae* reproduce more than the ones feeding on *T. latifolia* or *R. communis*, respectively (Park et al., 2011). Second, it was proven that the quantity of food also affects the fecundity rate where the intrinsic rate of increase averaged 0.14, 0.17 or 0.22 females/female/day as *A. swirskii* were feeding on 4, 8 or 12 eggs of *B. tabaci*/female/day, respectively (Fouly et al., 2011). Third, the environmental factors including temperature and RH play an affect the fecundity rate. As temperature increased from 15°Cto 35°C, the daily oviposition increased from 0.49 to 2.4 eggs/female/day (Nguyen, 2015). At 70% and 80% RH, 13 and 12 eggs/female/10 days were laid respectively while with 55% and 95% RH, 10 and 9 eggs/female/10 days were laid respectively (Nguyen, 2015).

c. Prey spectrum and feeding behavior

A. swirskii is a type III generalist predatory mite where it feeds on a broad spectrum of food. It feeds on thrips, whiteflies, several phytophagous mites, tetranychid, tarsonemid and eriophyid mites, in addition to other hemipteran insects and eggs of several lepidopterans. It also feeds on non-prey food source as pollen and honeydew. For example, cattail pollen (*T. latifolia*) was found to be a good food source for *A. swirskii* as it is used in laboratory rearing of the predatory mite. Goleva and Zebitz (2013) evaluated the development and reproduction of *A .swirskii* fed on pollen of 21 plant species. They classified them into 6 categories: (1) highly suitable: *Schlumbergera* hybrid, *Crocus vernus* Hill >*Echinocereus* sp. > *Paulownia tomentosa* (Thunb.) Steud., *Aesculus hippocastanum* Haynes, (2) suitable: *Ricinus communis* L. > *Betula pendula* Roth, *Zea mays* L., *Tulipa gesneriana* L. > *Abutilon* sp. > *Calla palustris* L., (3) ample suitable: *Cucurbita pepo* L. > *Pinus sylvestris* L. > *Narcissus pseudonarcissus* L., (4) bad: bee pollen, (5) negligible suitability: *Corylus avellane* L. > *Helianthus annuus* L. > Poaceae mix, and (6) not edible or toxic, resulting in 100 % mortality: *Lilium martagon* L., *Hippeastrum* sp. and *Hibiscus syriacus* L.

For mass rearing of *A. swirskii, Carpoglyphus lactis* L. (Acari: Carpoglyphidae) and *Thyreophagus entomophagus* (Laboulbene) (Acari: Acaridae) are being used as a food source (Nguyen,2015). *Suidasia medanensis* was found to be a better food source than *C.lactis* for delivering *A. swirskii* in sachets (Baxter et al., 2011).

Cannibalism and intraguild predation are expected to occur mainly when the preferred prey is scarce. The degree of cannibalism and intraguild predation varies between phytoseiids.

Abdel-Khalek and Momen (2009) reported that the mother *A. swirskii* did not eat neither eggs nor protonymphs but ate larvae at a very low frequency indicating that the rate of kin cannibalism is very low. Like cannibalism, intraguild predation takes place by *A. swirskii* when the preferred prey is low.

5. *Practical applications in biocontrol*

a. Target pests and crops

Amblyseius swirskii is commonly used in augmentative biocontrol to control whiteflies and thrips in greenhouse vegetables (cucumber, pepper and eggplant) and some ornamental crops (chrysanthemum, roses, gerbera) mainly in Europe and North America (Messelink et al., 2006). Several studies have been conducted to test the potential of *A. swirskii* as a biological control agent of agricultural pests. *A. swirskii* shows to be a potential predator of *B. tabaci.*

Nomikou et al. (2002) in his study reported that *A. swirskii* was able to suppress *B. tabaci* population growth in a cucumber greenhouse. The numbers of whiteflies on the plants without predators was significantly higher than on plants with predators with an average of 62-fold increase and 4 folds increase, respectively. Calvo et al. (2008) reported that *A. swirskii* was promising in controlling whitefly nymphs with release rate of 25 and 100 mites per m^2. Berndt et al. (2007) noted that *A. swirskii* successfully controlled *T. vaporariorum* on gerbera plants where whitefly larvae were no longer present six weeks after releasing the predatory mite.

Several studies have reported the effectiveness of *A. swirskii* as a biological control agent of thrips. Messelink et al. (2006) evaluated the effectiveness of ten phytoseiid predators in controlling *F. occidentalis* in greenhouse cucumber. *A. swirskii* controlled thrips significantly better than the other tested phytoseiids including the standard species *N. cucumeris.* Arthurs et al. (2009) evaluated the effectiveness of two phytoseiid species *N. cucumeris* and *A. swirskii* on sweet pepper. Although both species significantly reduced thrips number after 28 days of a single release (30 mites/plant), but *A. swirskii* was a more effective predator compared to *N. cucumeris* with 1 and 36 thrips per terminal leaf, respectively as compared with 70 for the control.

However, better control measures of Western flower thrips (*Frankliniella occidentalis*) and the greenhouse whitefly (*Trialeurodes vaporariorum*) were achieved in a system of 1 predator-2 prey system. The density of whitefly was reduced dramatically when both preys were present together while the density of thrips was reduced in the presence or absence of the whiteflies. As for the predator species, their densities were 15 times higher compared to their densities with only thrips or whiteflies (Messelink et al, 2008). Calvo et al. (2011) reported that 75 *A. swirskii* per m^2 were efficient in controlling *B. tabaci* and *F. occidentalis* pests either alone or simultaneously in cucumber greenhouses. This is due to a higher juvenile survival and developmental rate on a mixed diet of *A. swirskii.*

b. Commercial use and release strategy

The commercial production of *A. swirskii* started in 2005 as several biological control companies produced it including Koppert B.V. (Netherlands), Biobest N.V. (Belgium), and Syngenta Bioline Limited (UK).

According to their website, Koppert distributes *A. swirskii* under the product names Swirski-Mite, Swirski-Mite Plus and Swirki Ulti-Mite. Swirski Mite is a 500 ml bottle which contains 50,000 predatory mites (nymphs and adults) mixed with bran. Swirski-Mite Plus is a paper sachet which contains 250 predatory mites and storage mites mixed with bran with 100 or 500 sachets per box. Swirski Ulti-Mite is sachet with hook containing 250 predatory mites and storage mites mixed with carrier material or bran with 100 or 500 sachets per box. Biobest website commercializes *A. swirskii* under the product names Swirskii-Breeding-System and Swirskii-Long-Life-System. Swirskii-Breeding-System is breeding sachet which contains 250 predatory mites with bran and factitious prey. They are available in 100 or 500 sachets per unit. Swirskii-Long-Life-System is a sachet containing approximately 150 predatory mites with bran and factitious prey in a box with 500 sachets.

Syngenta Bioline produces *A. swirskii* under the name Swirkiline and Bugline swirskii. Swirskiline Loose is a 1 L cardboard tube with 25,000 predatory mites, while the Swirskiline Bulk is a 5 L bulk bags containing 125,000 predatory mites. Swirskiline sachets is a Gemini and Hooked sachets with a breeding colony of 250 mites at the time of packaging. Bugline Swirksi is a 6 m strip of sachets which is a unique and patented delivery system suited for crops that are grown in beds, rows or on a growing table. The predatory mites can be released directly on the crop through bran or vermiculite carried sprinkled on the leaves, released via sachets or broadcast via air blast. The recommended release rates are 25 and 100 mites per m^2 depending on the species and density of the pest and the crop type. 20 mites/ m^2 as a preventive measure and 100 mites/ m^2 for heavy thrips and whitefly infestations.

Slow release sachets (water resistant) allow gradual release of *A. swirskii* for several weeks through a small hole. Sachets with 150-250 mites can be suspended on the plant at a rate of 1 sachet/ 2m². To maintain continuous presence of the predatory mite, introduction should be repeated every 4 weeks.

To enhance the establishment and population growth of *A. swirskii* in fields with crops producing little or no pollen, food supplements can be distributed to enhance the survival of the predatory mite in the absence of its prey. Biobest produces and commercializes Nutrimite, a pollen product of *Typha angustifolia* L. pollen for this purpose. According to Biobest, Nutrimite should be sprayed using a Nutrigun at 500g/ha every 2 weeks.

I. Rearing of natural enemies

Natural enemies are mass reared in bio-factories to be released in large amounts for immediate pest control as an augmentative biological control measure. However, few commercial agricultural systems adopted this control measure because it is more expensive than the chemical pesticides (van Lenteren, 2012). Thus, a more cost-effective rearing techniques with reduced rearing cost are needed which might include factitious or artificial diets instead of their natural prey or hosts.

1. *Factitious foods*

Factitious prey are live, frozen, lyophilized or irradiated insects, mites and crustaceans that support the development and reproduction of predators or parasitoids instead of natural or target prey (hosts). Factitious food sources can be used as a preventative strategy to help establish or maintain a population of certain arthropod predators in the crop when pest

populations are low, to reduce the frequency of releases (Nguyen et al.,2014). Successful rearing methods of a predator on such a diet depends mainly on the predators' feeding habitat whether it is monophagous, oligophagous, or polyphagous. The latter have better chances of surviving and reproducing on factitious food sources (Riddick,2009).

Some lepidopterans eggs have been found to be nutrient rich food source for several generalist coccinellids (Riddick,2009). Fauvel et al. (1987) reported that the daily fecundity of *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) females reared on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs was approximately 3 or 8 times higher than that of females reared on *Aphis gossypii* Glover (Hemiptera: Aphididae) or *Tetranychus turkestani* Ugarov & Nycolsky (Acari: Tetranychidae), respectively. Specty et al. (2003) found out that the eggs of the Mediterranean flour moth *E. kuehniella* contained higher percentage of amino acids and lipids compared to the pea aphid *Acyrthosiphum pisum* (Harris) (Hemiptera: Aphididae), and that *E. kuehniella* eggs could support the growth and reproduction of the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) to a similar and better extent than the aphid. De Clercq et al. (2005) proved that adults of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) reared on live pea aphids, *A. pisum*, had better egg hatch compared to the ones reared on irradiated or frozen *E. kuehniella* eggs however they were only half as fecund as those offered *E. kuehniella* eggs. Maes et al. (2014) reported that larvae of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) developed faster on the eggs of *E. kuehniella* than on eggs of the mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), which is the predator's natural prey.

Cysts of the brine shrimp *Artemia sp*. (Anostraca: Artemiidae), a routinely used feed in aquaculture, are a non-insect type of factitious food which have been tested for rearing a number of insect predators. For rearing predatory arthropods, *Artemia cysts* is at advantage of *E. kuehniella* eggs because the market price of the former is lower than the latter and since it can be stored in a dry form for several years in cool and dry conditions without the need for deep freezing (Arjis and De Clercq, 2001). Arijs and De Clercq (2001) were the first to test cysts of the brine shrimp *A. franciscana* as a food for a heteropteran predator. They compared the development and reproduction of anthocorid *O. laevigatus* on *A. franciscana* cysts versus that on *E. kuehniella* eggs. The developmental and reproductive rates of the predators were similar for both diets whether feeding on hydrated decapsulated cysts or those feeding on frozen *Ephestia* eggs. A follow-up study by Bonte and De Clercq (2008) confirmed that brine shrimp cysts are suitable food for this anthocorid. Riudavets et al. (2006) tested the reproduction rate of the mirid *M. caliginosus* and the anthocorid *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae), feeding on dry cysts of *Artemia sp*. or frozen *E. kuehniella* eggs as food sources. The number of offsprings of *M. caliginosus* females was similar in both diets; however, the survival rate and numbers of offspring of *O. majusculus* fed on *E. kuehniella* eggs were higher than on *Artemia cysts*.

Castañé et al. (2006) found that *M. caliginosus* reared on dry or hydrated cysts of *Artemia sp.* had the same nymphal survivorship, nymphal developmental time, adult weight and fecundity as that obtained with *E. kuehniella* eggs; however, nauplii of the brine shrimp yielded a significant reduction in survivorship, a delay in nymphal development and a lower reproduction. Nguyen et al. (2014) tested *Ephestia kuehniella* Zeller eggs

(Lepidoptera: Pyralidae), decapsulated dry cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae), and meridic artificial diets (composed of honey, sucrose, tryptone, yeast extract, and egg yolk) supplemented with pupal hemolymph of the Chinese oak silkworm *Antheraea pernyi* (Gue´rin-Me´neville) (Lepidoptera: Saturniidae) (AD1), with *E. kuehniella* eggs (AD2) or with *A. franciscana* cysts (AD3). *A. swirskii* performed best on decapsulated *Artemia cysts.* Although the different factitious and artificial diets tested supported the development and reproduction *of A. swirskii* for a single generation but fitness losses occurred to a varying degree after several generations on *E. kuehniella* eggs or the artificial diets. The artificial diet enriched with *A. franciscana* cysts yielded better results than the other artificial diets. *Artemia cysts* are less expensive than *E. kuehniella* eggs and can support the development and reproduction of several predatory bugs; however prolonged rearing on the cysts as a sole food has been associated with fitness losses, at least in *Orius* bugs (De Clercq et al., 2005). Thus, in order to maintain a good food source, currently dry *Artemia cysts* are routinely mixed with *E. kuehniella* eggs for the production of different predatory heteropterans. This technique may help in reducing the cost of rearing system by reducing the amount of the expensive lepidopteran eggs and thus rationalize the production process of these economically important predators (De Clercq et al., 2014).

Several storage mites including *Carpoglyphus lactis* L. (Acari: Carpoglyphidae), *Thyreophagus entomophagus* (Laboulbene) (Acari: Acaridae), *Lepidoglyphus destructor* (Acari: Glyciphagidae) and *Suidasia medanensis* (Acari: Suidasiidae) are used as a primary food source in the mass production of different generalist phytoseiids instead of their

natural prey (Bolckmans et al., 2012). Schliesske (1981) tested *Tyrophagus casei* (Oudemans) (Acari: Acaridae) as alternative prey for *N. cucumeris* and *Neoseiulus barkeri* (Hughes) (Acari: Phytoseiidae). *T. casei* proved to be a good alternative food source as both phytoseiid mites were successfully able to develop and reproduce on it. Bolckmans et al. (2012) found out that total fecundity of the females fed on juvenile stages of *C. lactis* were similar as that on adult stages of *C. lactis* with 1.80eggs/female/day and 29 eggs over 16 days versus 1.84 eggs/female/day and 33 eggs over 18 days, respectively. They also found out that *A. cucumeris* was successfully able to reproduce on *C. lactis* with 2.13 eggs/female/day. Fidgett and Stinson (2008) concluded that when *A. cucumeris* was reared on *T. entomophagus,* the population of the former increased 10 folds within 14 days which is double the value achieved for *A. cucumeris* in commercial production on diet of *Tyrophagus putrescentiae* Schrank (Acari: Acaridae). They also reported that both *T. entomophagus* and *C. lactis* are good food source for *A. swirskii*, but the population of the predator which was feeding on *T. entomophagus* was 38% greater than that feeding on *C. lactis.*

2. *Artificial diet*

The utilization of an artificial diet may be the next step towards a more cost-effective rearing of predators. It reduces the complexity of the system to more manageable levels. Artificial diets help in rapid development of arthropod parasitoids or predators without the time lag required to build up host or prey populations. Moreover, by using artificial diets, risks of diseases which might occur to the host, prey or plant culture are avoided. King et al. (1985) considered that it is the development of artificial diets that will contribute to the

increase of augmentative biological control, and Cohen (2015) indicated that the greatest barrier of mass production of entomophagous insects is the absence of a suitable artificial diet.

Several terms are used to describe types of artificial diet. Dougherty (1959) classified artificial diets into holidic, meridic and oligidic according to the ingredients used (Nguyen, 2015). “Holidic diets pertain to media whose intended constituents, other than purified inert materials, have an exactly known chemical structure before compounding. Meridic diets pertain to media composed of a holidic base to which is added at least one substance or preparation of unknown structure (for example, protein, regardless of “purity”) or of uncertain purity. Oligidic diets pertain to media in which crude organic materials supply most dietary requirements” (Nguyen Duc,2015). However, the distinction between these three classifications has not always been clear; for this reason, other classification systems were suggested. Grenier and De Clercq (2003) classified artificial diets according to the presence or absence of insect components (i.e., tissues, hemolymph, cells, protein, amino acids, etc.). While Parra (2012) classified artificial diets based on formulation: (1) diets as powder or fragments, (2) semiliquid diets for chewing phytophages, (3) liquid diets for sucking phytophages, or (4) liquid diets for endoparasitoids.

a. <u>Function of diet components:</u>

A diet must provide all essential nutrients to allow an arthropod to complete its development and reproduction. Several main ingredients are included in artificial diets for arthropods including a nitrogen source (e.g. protein, free amino acids), carbohydrates, lipids, vitamins, minerals and water. More ingredients like stabilizers, preservatives,

"fillers" or bulking agents are added to the diet to help in preserving its structure. "Token stimuli" which stimulate the feeding responses without having direct nutritional function are added to artificial diets and therefore making them successful diets.

Proteins are the source of amino acids that are needed by the insect to produce tissues and enzymes. Arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine are essential amino acids for the normal growth of arthropods. Other amino acids are essential by other species where glycine for some dipterous insects, alanine for *Blattella* cockroaches and proline for *Phormia* blowflies. It is important to have a good balance between the different amino acids as the protein plays an important role in egg production (Chapman, 1998).

Lipids including fatty acids, phospholipids and sterols are components of cell walls. Although lipids are not usually essential dietary constituent since insects are capable of synthesizing several fatty acids and phospholipids, however some insects require a dietary source of polyunsaturated fatty acids, and all insects require sterols (Chapman,1998).

Carbohydrates are insects' fuel. Although they are important components in insects' diet since they may be converted to fats and may contribute to amino acid production, however they are not essential since they can be also synthesized from fats or amino acids (Chapman, 1998). Most insects are capable of absorbing and metabolizing fructose and glucose, however, some monosaccharides (ie. arabinose, ribose, xylose, and galactose) are readily absorbed but not metabolized. Generalist feeders like herbivores and predators are capable of digesting disaccharides, such as sucrose and maltose, while some parasitoids and

mites are not (Cohen,2015). Thus, it is worth saying that the requirement of carbohydrates in the insects' diet is species dependent.

Vitamins are classified as hydrophilic or hydrophobic depending on their solubility in water. Vitamins are usually required for growth in insects, which are unable to synthesize certain ones (Chapman,1998). Water soluble vitamins (hydrophilic) include vitamin C and B complex which is made up of thiamin (B_1), riboflavin (B_2), niacin = nicotinamide(B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9), and cobalamins (B_{12}). Vitamin B functions as cofactors for enzymes and are required in the diet of all insects (Chapman, 1998), except for vitamin B12, which is not universally required (Cohen, 2015). Vitamin C (ascorbic acid) is known to play a role in the molting process (molting is the shedding of the outer exoskeleton cuticle of insects and other arthropods during growth or in their transition from one life stage to another) (Chapman, 1998). However, lipid soluble vitamins (hydrophobic) include retinol, carotenoids (A), tocoferols (E), calciferol (D), and phyloquinone (K). Vitamin A and E are the only hydrophobic vitamins that are known to be required in insects, since they play a role in the synthesis of pigments and in reproduction, respectively (Chapman, 1998).

Vitamins C, A, and E are also antioxidants and may play an important role in the detoxification processes and protection against microbial infection (Cohen, 2015).

Minerals are essential elements for growth and reproduction; however, they cannot be biosynthesized meaning that they should be obtained from inorganic sources (Nguyen, 2015). Minerals are compounds that consist of combinations of cations and anions. Sodium,

potassium, calcium, magnesium, chloride and phosphate are required in insects' diet since they play a role in the functioning of insect cells (Chapman,1998). Iron is important in several enzyme pathways including in the synthesis of DNA (Cohen,2015). Zinc and manganese are also essential as they are included in the process of hardening the cuticle in many insects (Chapman,1998).

Water is the most important nutrient as it plays a role in all life processes. Water is taken by the insects from their food or drinking source; however, there is only a small group that can use their metabolic activity to create water by themselves. The water content of an insect diet is a crucial factor where low water content in the artificial diet will not provide enough water for the insect or cause difficulty in food intake, while high water in the artificial diet may encourage microbial contamination. Cohen (2004) suggested that the normal amount of water present in the artificial diet should be the same as that in the insect's natural food.

b. Artificial diet for phytoseiid mites

Although several articles on artificial diets for insect predators are present in the literature, few articles about rearing predatory mites on artificial diets exit and most of which are on very small laboratory scale.

The following media were successfully used for rearing *A. swirskii*:

1- Abou-Awad et al. (1992) successfully composed an artificial diet (yeast, milk, cysteine, proline, arginine, sucrose, glucose, streptomycin sulphate and sorbic acid) that allowed the successful development and reproduction of the predacious mites *Amblyseius gossipi* El-Badry and *A.swirskii.* However, compared to the species

feeding on natural prey, the fecundity of both species was lower although the eggs were normal.

2- Nuyen et al. (2014) evaluated the development, reproduction, and survival of A. swirskii on different diets: cattail pollen (*Typha latifolia* L.), dried fruit mite (*Carpoglyphus lactis* L.), or on two artificial diets, (AD1) composed of honey, sucrose, tryptone, yeast extract, and egg yolk and (AD2) composed of AD1 enriched with by hemolymph from oak silkworm pupae (*Antheraea pernyi* (Gue´rin-Me´neville)). *C. lactis* and AD2 were reported to have shorter immature and pre-oviposition periods and high intrinsic rate of increase (rm). The total number of deposited eggs was significantly higher for females fed on AD2, thus indicating the potential use of artificial diets for use in the mass production of the predator.

3- Nguyen et al. (2014) tested the reproduction and development ability of *A. swirskii* on different diets: *Ephestia kuehniella* Zeller eggs (Lepidoptera: Pyralidae), decapsulated dry cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae), and on meridic artificial diets (composed of honey, sucrose, tryptone, yeast extract, and egg yolk) supplemented with pupal hemolymph of the Chinese oak silkworm *Antheraea pernyi* (Gue´rin-Me´neville) (Lepidoptera: Saturniidae) (AD1), with *E. kuehniella* eggs (AD2) or with *A. franciscana cysts* (AD3). Different factitious and artificial diets supported the development and reproduction of *A. swirskii* for a single generation only as fitness losses were recorded after several generations on *E. kuehniella* eggs or the artificial diets. Among the artificial diets, the one enriched with *A. franciscana* cysts yielded better results than the others. *A.*

swirskii performed best on decapsulated Artemia cysts which indicates that it can be used in sustaining the population of the predator.

Other media used for rearing other phytoseiids:

1- McMurtry and Scriven (1966) tested the developmental time and oviposition rate of four phytoseiids (*Amblydromalus limonicus* Garman and McGregor, *Amblyseius hibisci* (Chant), *Typhlodromus occidentalis* Nesbitt and *Typhlodromus rickeri* (Chant) (Acari:Phytoseiidae) reared on various artificial diets including sucrose, molasses, yeast + sucrose or yeast+ molasses and compared with the ones reared on mite prey and pollen as food sources. Longer developmental times and lower oviposition rates were reported for the four phytoseiids reared on artificial diets compared with the one reared on mite prey and pollen.

2- Shehata and Weismann (1972) reported that *P. persimilis* failed to produce viable eggs when reared on three artificial diets although the larvae successfully developed into an adult.

3- Kennett and Hamai (1980) reported that seven out of nine predaceous mites (*A. hibisci*, *A. limonicus*, *Amblyseius largoensis* (Muma),*Metaseiulus pomoides* Schuster & Pritchard, *T. occidentalis*, *Typhloseiopsis arboreus* (Chant), *Typhloseiopsis pyri* Scheuten, *P. persimilis*, and *I. degenerans*) achieved complete development and oviposition when fed on artificial diet which consisted of bee honey, sugar, yeast flakes, yeast hydrolysate, enzymatic casein hydrolysate and

fresh egg yolk. However, the oviposition rate of all nine species were lower than the ones feeding on their natural prey.

4- Shih et al. (1993) evaluated responses of *Euseius ovalis* (Evans) to natural food resources and two artificial diets (diet A) consisted honey 15 g, ascorbic acid 100 mg, vitamin B complex 300 mg, Bovril (a commercial beef extract) 500 mg, choline chloride 0.1 g, and distilled water 120 mL; and diet B consisted D (-) fructose 10 g, fresh egg yolk 20 g, yeast powder 10 g, honey 10 g, choline chloride 0.1 g, distilled water 180 ml). Although immatures developed in the first generation, offspring was not able to complete its life cycle when maintained on the artificial diets. Moreover, shorter oviposition period, lower daily and total reproductive rates were observed by *E. ovalis* females that were maintained on artificial diets.

5- Ogawa and Osakabe (2008) tested the development and survival of *Neoseiulus californicus* (McGregor) on an artificial diet composed of honey, sucrose, tryptone, yeast extract, fresh egg yolk, and distilled water. *Neoseiulus californicus* were able to develop successfully, but few eggs were deposited. Although phytoseiid mites can develop on different artificial diets, fecundity in most cases was inferior to that on natural or factitious prey.

3. *Pollen*

Pollen is a good source for several nutrients that are essential for the development and reproduction of entomophagous arthropods. It provides proteins, free amino acids, carbohydrates, lipids, vitamins, flavonoids, and minerals. Since the protein and oil content present in pollen is qualitatively and quantitatively superior to most vegetative tissues and

even many prey items, many insects that don't consume plant tissues eat pollen (Lundgren,2009).

Phytoseiidae, Erythraeidae, Cheyletidae and Stigmaeidae are families of predatory mites that feed on pollen to different extents. The feeding behavior of phytoseiid predatory mites is classified into four groups as mentioned previously where only Type I, specialized predators of *Tetranychus* species, do not feed on pollen, whereas type II, III and IV predatory mites are found to be more or less feeding on pollen. In Type II, pollen promotes the reproduction in some species, but at a lower extent than its natural prey. Many species that belong to Type III were reported to feed on pollen as some species belonging to that group have reproduction rate as high as feeding on the natural prey. Type IV specialized pollen feeders comprises only the genus *Euseius*; members of this group (e.g. *Euseius tularensis* (Congdon) (Acari: Phytoseiidae)) can subsist on pollen in the absence of prey with minimal reduction in fitness (Nguyen ,2015). The genus *Neoseiulus* including *N. californicus, Neoseiulus fallacis, Neoseiulus idaeus, Neoseiulus longispinosus* (Acari: Phytoseiidae) are considered to be the species that feed mostly on pollen (Nguyen Duc,2015).

In biological control practices and in order to avoid cannibalism when the prey is scarce, several species of predatory mites are provided with pollen as a supplementary or alternative food source. Through using banker plants, artificial pollen sources (plastic cups with pollen placed between the crop plants) or by dusting or spraying pollen from various plants (cattail, castor bean, and maize), pollen can be supplied as an additional food source.

Findings from literature demonstrated the need to work or research new innovative types of diets which could potentially improve and increase the population and fitness of insects.

CHAPTER III

MATERIALS AND METHODS

A. Collection and identification of local *Amblyseius swirskii*

During spring 2018, *A. swirskii* specimens, suspected to be local, were collected from a castor bean tree from Batroun, North Lebanon. Samples were sent to two specialized labs in mite identification, in Canada and Syria, for confirmation of identification based on morphological characteristics, molecular identification was done at the plant pathology lab at FAFS – AUB. The specimens were compared with a reference strain of *A. swirskii* supplied from Biobest.

B. Mass rearing of *Amblyseius swirskii*

1. Evaluation of rearing media for mass production of Carpoglyphus lactis

a. Maintaining *Carpoglyphus lactis* population

A colony of *C. lactis* was reared in an incubator at 25 ± 1°C, 70 ± 5% RH and a 16:8 h (L:D) photoperiod. Mites were reared in a round plastic container of diameter 10 cm and depth 4 cm containing different food sources. The containers with *C. lactis* were placed together with a similar container containing water to provide moisture at an adequate RH. Both were placed in a container (24cm x 14cm x 5.5cm) with a mesh hole in the lid for aeration (Figure 5).To deter mites from escaping, the edges of the container of all the experiments conducted were covered with Vaseline and the Containers were placed in a bigger tray containing 2 cm of water and soap.

Figure 5. Rearing compartments for *Carpoglyphus lactis*

b. Counting of *Carpoglyphus lactis*

After mixing the culture media well to homogenize the mix, around 50 mg of the mix was weighted and placed in an Eppendorf tube. 500µl distilled water was added to the Eppendorf tube which contains the mix. After shaking well, 20 µl sample were pipetted and placed on a petri dish. The solution was distributed along the Petri dish to help in spreading of *C. lacis* for better counting (Figure 6).To deter the mites from escaping, Vaseline was spread on the edges of the Petri dish so in case the mite tried to escape the dish, it will get stuck on the Vaseline.

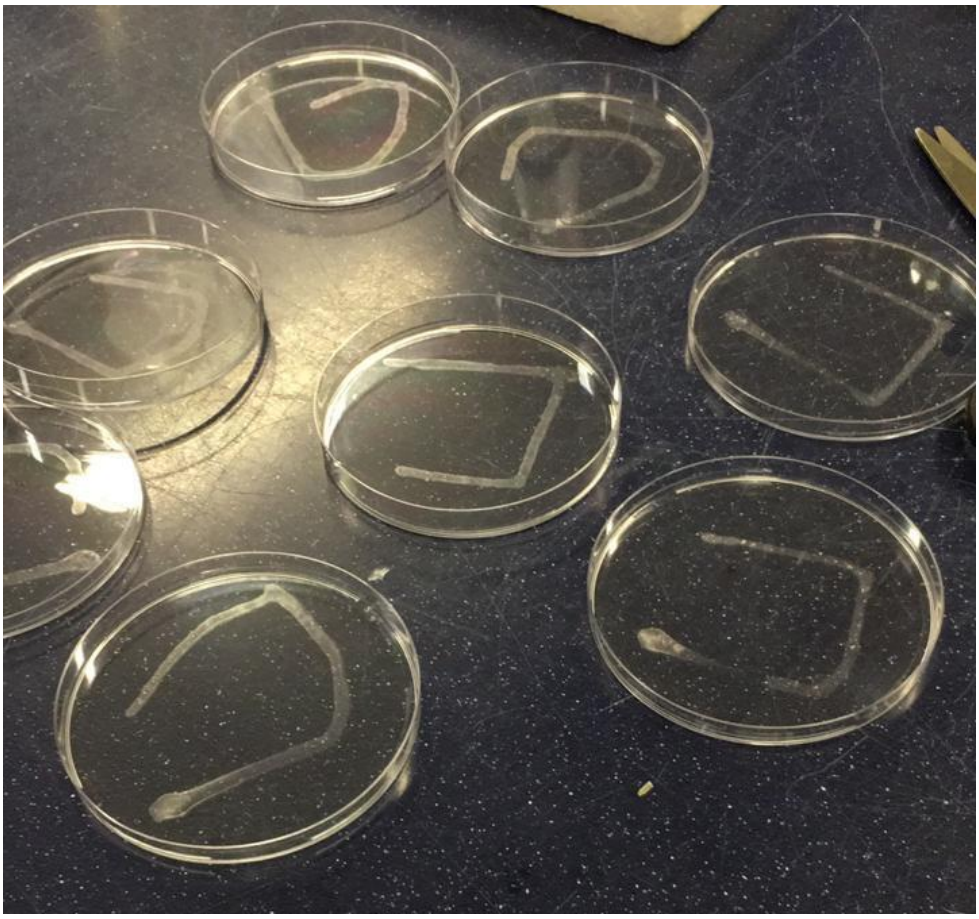


Figure 6. Preparation of samples for counting *Carpoglyphus lactis*

c. Evaluation of composition of food media for rearing *Carpoglyphus lactis*

i. Trial 1

In rectangular container (24cmx14cmx5.5cm), six different media were compared each containing 20g culture media + 4,000 *C. lactis* to determine which medium is the best for rearing *C. lactis*. Results were recorded weekly until the population of *C. lactis* started to decline.

A: 16g (80%) Berghol + 4g (20%) Yeast + 4,000 *C. lactis*

B: 20g Berghol + 4,000 *C. lactis*

C: 20g Yeast + 4,000 *C. lactis*

D: 20g Wheat Bran (P) + 4,000 *C. lactis*

E: 16g (80%) Wheat Bran (P) + 4g (20%) Yeast + 4,000 *C. lactis*

F: 16g (80%) Wheat Bran (P) +4g (20%) Berghol + 4,000 *C. lactis*

ii. Trial 2

Similar to Trial 1 but one additional medium was added to check if additional quantity of yeast from 20% to 30% would extend the lifespan of *C.lactis.*

G: 14g (70%) Wheat Bran + 6g (30%) Yeast + 4,000 *C. lactis*

iii. Trial 3

To determine the population dynamic of *C. lactis*, two treatments were tested one without and one with 15% yeast. 75,240 *C. lactis* were added to 50g of autoclaved coarse wheat bran and placed in a rectangular Container (28cm x 18cm x 5cm). Results were recorded weekly until the population of *C. lactis* started to decline.

C: 50g Wheat Bran (C) +15% Yeast +75,240 *C. lactis*

D: 50g Wheat Bran (C) +75,240 *C. lactis*

iv. Trial 4

After finding out that yeast is essential for the development of *C. lactis*, different yeast ratios along with different rates of *C. lactis* were tested to find out the best medium for

mass rearing *C. lactis*. 50g autoclaved coarse wheat bran were added to a Container (28cm x 18cm x 5cm) with a combination of 10% or 20% yeast along 37,500 or 150,000 *C. lactis*. Results were recorded weekly.

A: 50g Wheat bran(C) + 5g yeast (10%) + 37500 *C. lactis*

B: 50g Wheat bran(C) + 5g yeast (10%) + 150000 *C. lactis*

C: 50g Wheat bran(C) + 10g yeast (20%) + 37500 *C. lactis*

D: 50g Wheat bran (C) + 10g yeast (20%) + 150000 *C. lactis*

2. Evaluation of rearing media for mass production of Amblyseius swirskii

a. Maintaining *Amblyseius swirskii* populaion

Preliminary research data were based on a stock colony of *A. swirskii* that was initiated from specimens supplied by Biobest N.V. and was kept in an incubator at 25 ± 1°C, 70 ± 5% RH and a 16:8 h (L:D) photoperiod. Later on, all, the experiments were conducted with the local strain. Mites were reared on yeast in Container (24cm x 14cm x 5.5cm) with a mesh hole in the lid. A small piece of sewing thread was placed to serve as an oviposition substrate. A small round plastic container was placed inside the Container to provide humidity of 70 ± 5%. To prevent mites from escaping, the edges of the Container were covered with Vaseline and the Container were placed in a tray with 2 cm of water and soap (Figure 7).

Figure 7. Rearing compartment for *Amblyseius swirskii*

b. Counting of *Amblyseius swirskii*

After mixing the medium well to homogenize the mix, 50 mg culture media are sampled with a minimum of 5 replicates per treatment and placed in a petri dish sealed with Vaseline to prevent mites from escaping. Under a stereo microscope, alive *A. swirskii* are counted and thus calculated accordingly to determine the number of SW (*Amblyseius swirskii*) in the original medium.

c. Evaluation of composition of food media for rearing *Amblyseius swirskii*

Several experiments were conducted to determine the most effective media to rear large numbers of the predatory mite in a feasible way.

Experiments 1, 2 and 3 were conducted in a Container (28cm x18cm x 5 cm). Experiments 4 and 5 were carried out in a round plastic container of diameter 10 cm and depth 4 cm which was placed in a bigger plastic placed Container (28cm x 14cm x 5.5cm) All

containers used for rearing *A. swirskii* had a mesh hole in the lid for aeration. To provide humidity of 70 ± 5%, a round plastic container of diameter 10 cm and depth 4 cm containing distilled water was placed in each treatment.

All trials for rearing *A. swirskii* were conducted in an incubator at 25 ± 1°C, 70 ± 5% RH and a 16:8 h (L:D) photoperiod.

i. Trial 1

In order to determine the best combination of *A. swirskii*, *C. lactis, and* yeast, the following experiment was conducted as a preliminary study. 1:75 of *A. swirskii*: *C. lactis* were tested in the absence or presence of 15% yeast. Another treatment of 1:150 was tested without the addition of yeast to check if the high population of *C. lactis* can overcome the need of adding yeast. 50g autoclaved coarse wheat bran were added to each Container as follows:

A: 50g Wheat Bran (C) + 15% Yeast + 1,000 *A. swirskii* + 75,000 *C. lactis*

B: 50g Wheat Bran (C) + 1000 *A. swirskii* + 75,000 *C. lactis*

F: 50g Wheat Bran (C) + 1000 *A. swirskii* + 150,000 *C. lactis*

ii. Trial 2

As a preliminary study to determine the best ratio of *A. swirskii*, *C. lactis, and* yeast. 50 g of autoclaved coarse wheat bran were added to a Container (28cm x 18cm x 5cm) with 0%, 10% and 20 % yeast in each treatment. 1,500 *A. swirskii* were added and the numbers of C. lactis was adjusted to get the following final ratios of A. swirski to C. lactis of 1:50, 1:100, and 1:200, respectively. The needed numbers of *C. lactis* to be added to each treatment

75,000- 150,000- 300,000 *C. lactis* were added to the 1:50, 1:100 and 1:200, respectively. Numbers of both *A. swirskii* and *C. lactis* were recorded weekly.

The following 9 treatments were compared:

A: 50g Wheat Bran (C) + 1,500 *A.swirskii* +75,000 *C. lactis*

B: 50g Wheat Bran (C) + 1,500 *A.swirskii* + 150,000 *C. lactis*

D: 50g Wheat Bran (C) + 1,500 *A.swirskii* + 300,000 *C. lactis*

E: 50g Wheat Bran (C) + 10% yeast + 1,500 *A.swirskii* +75,000 *C. lactis*

F: 50g Wheat Bran (C) + 10% yeast + 1,500 *A.swirskii* +150,000 *C. lactis*

H: 50g Wheat Bran (C) + 10% yeast +1,500 *A.swirskii* + 300,000 *C. lactis*

I: 50g Wheat Bran (C) + 20% yeast + 1,500 *A.swirskii* + 75,000 *C. lactis*

J: 50g Wheat Bran (C) + 20% yeast + 1,500 *A.swirskii* + 150,000 *C. lactis*

L: 50g Wheat Bran (C) + 20% yeast +1,500 *A.swirskii* + 300,000 *C. lactis*

iii. Trial 3

The promising treatments from Trial 2 were tested again. 50g autoclaved coarse wheat bran were added to a Container (28cm x 18cm x 5cm) with 10% and 20% yeast. 750 *A. swirskii* were added along with 37,500 and 150,000 *C. lactis* to obtain a ratio of 1:50 and 1:200 respectively. Control for each treatment was carried out in the absence of *A. swirskii* to check the population dynamic of *C. lactis*. Results were recorded weekly until the population of *A. swirskii* started to decline.

E: 50g Wheat bran (C) + 5g yeast (10%) + 37500 *C. lactis* + 750 *A. swirskii*

F: 50g Wheat bran (C) + 5g yeast (10%) + 150000 *C. lactis* + 750 *A.swirskii*

G: 50g Wheat bran (C) + 10g yeast (20%) + 37500 *C. lactis* + 750 *A.swirskii*

H: 50g Wheat bran (C) + 10g yeast (20%) + 150000 *C. lactis* + 750 *A.swirskii*

iv. Trial 4

Two treatments, 2 replicates each, were tested to check the effectiveness of doubling the volume in extending the production cycle of *A. swirskii*. Trials were run in rounded plastic containers 10 cm in diameter and depth 4cm (20%) yeast was added to each treatment. 10g autoclaved coarse wheat bran were compared with 20g autoclaved wheat bran. Two replicates were made of each of the following treatments:

A: 10g Wheat Bran(C) +2g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* (1:50 RATIO

B: 10g Wheat Bran(C) +2g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* (1:50 RATIO)

C: 20g Wheat Bran(C) +4g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* (1:50 RATIO)

D: 20g Wheat Bran(C) +4g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* (1:50 RATIO)

v. Trial 5

Two treatments with 2 replicates each were evaluated to check whether spraying calcium propionate intended to prevent infection of the culture medium by fungi will help in providing moisture to the media as an alternative form of placing water inside the rearing container. Trials were run in rounded plastic containers 10 cm in diameter and depth 4 cm.

10g autoclaved coarse wheat bran were added to 20% yeast (2g). To provide humidity, a water container was placed beside the container used for rearing *A. swirskii* in the control while in the other treatments, humidity was provided through spraying calcium propionate every 4 days in order to make use of the entire rearing space inside the Container. Two replicates were made of each of the following treatments:

A: 10g Wheat Bran(C) +2g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* (1:50 RATIO

B: 10g Wheat Bran(C) +2g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* (1:50 RATIO)

E: 10g Wheat Bran(C) +2g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* but spraying calcium propionate every 4 days

F: 10g Wheat Bran(C) +2g Yeast (20%) +300 *A. swirskii* +15,000 *C. lactis* but spraying calcium propionate every 4 days

vi. Trial 6

To check for the effectiveness of sucrose in rearing *A. swirskii,* 3 g sucrose were added to a round plastic container of diameter 10 cm an 4 cm height in the presence of 10g autoclaved coarse wheat bran, 2 g yeast (20%) , 300 *A. swirskii* and 15,000 *C. lactis* to reach a ratio of 1:50. As a control, all of the previously mentioned ingredients were added except for sucrose. Results were recorded weekly until the population of *A. swirskii* started declining.

A: 10g Wheat Bran +2 g Yeast +300 *A. swirskii* +15,000 *C. lactis*

B: 10g Wheat Bran +2 g Yeast +300 *A. swirskii* +15,000 *C. lactis*

C: 10g Wheat Bran +2 g Yeast +3 g sucrose +300 *A. swirskii* +15,000 *C. lactis*

D: 10g Wheat Bran +2 g Yeast +3 g sucrose +300 *A. swirskii* +15,000 *C. lactis*

3. Statisical analysis

The trial design was a factorial arrangement of treatments in a Complete Randomized Design. Data was analyzed using the General Linear Model (GLM) and means were compared using T-test at P level of 0.05. Significant interactions were followed by One Way ANOVA and Tukey's test for mean separation. Statistical analysis was performed using IBM SPSS statistics (SPSS v. 25).

C. Small scale experiment for evaluating the efficacy of local *Amblyseius swirskii* in controlling whiteflies

To check the efficacy of *A. swirskii* in controlling whiteflies on cucumber at two different release rates, three treatments were tested: Control with only whiteflies, 50 *A. swirskii* /m² and 100 *A. swirskii* / m².

A total of nine small insect- proof cages were used. Every three cages were replicates for one treatment. Three plants at the 4- true leaf stage were placed in each cage. Four whitefly releases were done;15 whitely adult were released each of week 0 and 1 and 30 whitefly adults each of week 2 and 4 in all three treatments.50 and 100 SW adults were released in treatments 1 and 2 respectively at week 1. (Table 1) and results were compared to the untreated control.

Table 1.The release schedule of *B. tabaci* and *Amblyseius swirskii*

	B. tabaci	*A. swirskii*

	Untreated Control	T1	T2	Untreated Control	T1	T2
Week 0/ Feb 27	15	15	15	0	0	0
Week 1/ March 6	15	15	15	0	50	100
Week 2/ March 13	30	30	30	0	0	0
Week 4/ March 27	30	30	30	0	0	0

Figure 8. Photo of an insect-proof cage where the cucumber seedlings were put for the evaluation of the efficacy of *A. swirskii* against *B. tabaci*

1. Statistical analysis

Data were analyzed using one-way ANOVA and means were compared using T-test at P level of 0.05. Statistical analysis was performed using IBM SPSS statistics (SPSS v. 25).

D. Large scale greenhouse experiments for evaluation of the efficacy of *Amblyseius swirskii* for the management of two greenhouse cucumber pests: whiteflies and thrips in Kfarmashoun/Caza of Byblos

1. Experimental site and transplanting

The experiment was conducted in Kafarmashoun, Byblos Caza, North of Lebanon (300 m altitude), starting May 27, 2019. Two greenhouses were selected; one used as a control where the farmer followed his normal production and protection practices; while in the second one, IPM measures were implemented. The area of each arched greenhouse was 450 m^2 (50m*9m*). The transplanting of cucumber seedlings "SERINA" variety at the 2-3 true leaf stage took place during the first week of March 2019 at a rate of 2 cucumber seedlings per m^2 on 10 rows. Pepper plants "TALA" variety were already present on the boarder rows of the greenhouse from the previous season. A total of 1000 cucumber plants, and 200 pepper plants were present in each greenhouse (Figure 9).

Figure 9. Kfarmashoun greenhouse (control) where the cucumber seedlings were transplanted.

2. Pre-transplanting measures in IPM greenhouses:

Data loggers (Ebro®) were installed to record daily temperature and RH, at an interval of 15 minutes. For the elimination of recently introduced whiteflies, thrips and fungus gnats from the IPM greenhouses, yellow sticky cards and blue sticky cards (BSCs), provided by the Ministry of Agriculture extension service, were installed at rate of 1 trap/16 m² (Figure 10). Moreover, in the IPM greenhouse, trap/habitat plants including marigolds (Figure 11) and alyssum (Figure 12) were planted at a rate of 1 flowering plant per around 20 m^2. The trap/habitat plants were replaced whenever a high number of insects were found feeding on them. Weeds, inside and around the greenhouses were pulled by hands instead of applying

herbicides. For nematodes and as a preventive measure, the farmer had treated the soil with Imyciaphos (Nemakick) via drip irrigation.

Figure 10. Photo showing yellow and blue sticky cards in IPM greenhouse.

Figure 11. Photo showing marigold flower in the IPM greenhouse.

Figure 12. Photo showing alyssum plant in IPM greenhouse.

3. Pre-transplanting measures in control greenhouse

In the control greenhouse, nematode and weed management strategies were similar to that of the IPM greenhouse. Two blue sticky traps were installed one at the entrance near the door and the second one at the end.

4. Post-transplanting measures

a. Insect/mite scouting

Scouting for insects/mites was performed weekly in both greenhouses. A total of 50 cucumber plants were scouted, five plants from each row and 30 pepper plants, 15 plants from each row. From each plant, three leaves: upper, middle and lower leaves were scouted. The number of aphids, adults and nymphs of whiteflies, thrips and spider mites

were recorded weekly in both greenhouses. The same scouting technique was used also for monitoring populations of the released natural enemies; *A. swirskii* and *P. presimilis* in the IPM greenhouse only (Figure 13).

Figure 13. Photo showing scouting cucumber leaves for pests and predators.

b. Natural enemy introductions

During the experimental period, 7 and 10 introductions of *A. swirskii* and *P. presimilis* were done respectively (Table 2). *A. swirskii* were sprinkled with bran carrier from a bottle on cucumber and pepper leaves(Figure 14,Figure 15). However, bean leaves containing *P. presimilis were* distributed on both cucumber and pepper plants. *Beauveria bassiana* of concentration $10^{\hat{}8}$ was sprayed on pepper hotspots only as a measure of controlling mainly aphids on pepper plants.

Figure 14. Photo showing releasing of *Amblyseius swirskii* by means of wheat bran carrier on pepper leaves

Figure 15. Photo showing *Amblyseius swirskii* release by means of a carrier from a bottle on cucumber leaves.

Table 2. Dates of introduction and release rates of natural enemies in the IPM greenhouse

Date	Week	*Amblyseius swirskii*	*Rate of A. swirskii/m²*	*Phytoseiulus persimilis*	*Beauveria bassiana*
March 27,2019	0	16,000	36	10,000	
April 3, 2019	1	8,000	17	200	
April 10 ,2019	2	27,000	60	1,000	10ˆ8 on hotspots
April 17 ,2019	3	-	-	-	-
April 24,2019	4	-	-	-	-
May 1,2019	5	15,000	34	-	10ˆ8 on hotspots
May 8,2019	6	26,000	58	700	10ˆ8 on hotspots

May 15,2019	7	10,000	23	1,000	
May 22,2019	8	5,000	11	1,000	
May 28,2019	9	-	-	5,000	
June 3,2019	10	-	-	500	
June 11,2019	11	-	-	2,000	
June 14,2019	12	-	-	9,000	
June 18,2019	13	-	-	-	

c. Pesticide sprays

vii. IPM greenhouse

The experiment started three weeks after transplanting of the cucumber seedlings. By that time the farmer had already applied two pesticide sprays on cucumber and pepper in both greenhouses. Later on, no insecticide/acaricide sprays were performed in the IPM greenhouse except but only three sprays with the fungicide chlorthalonil were applied as a preventive method to protect against infections by downy and powdery mildews. (Table 3).

viii. Control greenhouse

In the control greenhouse, during the growing season a total of 14 pesticide sprays with no correspondence with a scouting threshold were applied with pesticide mixtures containing a minimum of 2 active ingredients per mix (Table 3**Error! Reference source not found.**). Fungicide sprays were done to lower incidence of downy and powdery mildews while insecticidal/acaricidal sprays were done mainly to control thrips, whiteflies and spider mite.

Table 3. Details of pesticides applied in the control greenhouse throughout the growing period of cucumber plants.

Date	Week	IPM	Control
Before March 27,2019	0	2 sprays	3 sprays
		Emamectin benzoate	Emamectin benzoate
		Acetamiprid	Acetamiprid
		Fenbutatin oxide	Fenbutatin oxide
April 3, 2019	1		Zoral: Mancozeb, Cymoxanyl
			Acetamiprid
			Flonicamid
			BT
April 10 ,2019	2		Acetamiprid
			Tolfenpyrad
April 17 ,2019	3		
			-
April 24,2019	4	Chlorthalonil	Chlorthalonil
May 1,2019	5		2 sprays:
			Abamectin
			Thiomethoxam Acetamprid
			Tolfenpyrad
May 8,2019	6		Thiomethoxam
			Acetamprid
May 15,2019	7	Chlorthalonil	Mancozeb

May 22,2019	8	Chlorthalonil	Acetamiprid Carbosulfan Acetamprid Tolfenpyrad Abamectin
May 28,2019	9		-
June 3,2019	10		Mancozeb Cymoxanil Thiomethoxam
June 11,2019	11		2 sprays: Acetamprid Tolfenpyrad Abamectin Thiomethoxam Acetamiprid

5. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics (SPSS v. 25). The evolution of the average number of *A. swirskii* vs thrips adults, thrips larvae , whiteflys adults or whitefly nymphs per three leaves on cucumber and pepper plants in the control greenhouse and the biological control greenhouse were analyzed using one way ANOVA and means were compared using T-test at P level of 0.05.

CHAPTER IV

RESULTS & DISCUSSION

A. Collection and identification of local *Amblyseius swirskii*

Morphological identification by two separate specialized labs (Canada and Syria) showed that the samples of *Amblysieus* collected from Lebanon and those imported from Biobest are *Amblyseius swirskii*. The morphological identification was also confirmed by molecular identification conducted in Lebanon, which showed that the collected culture from Batroun and the Belgian Biobest cultures are both *Amblyseius swirskii* with 99% nucleotide similarity in the ITS gene as compared to the *Amblyseius swirskii* cultures reported at NCBI (add accession numbers).

The ITS nucleotide sequence (599 nucleotides) of the Lebanese strain of *Ambyseius swirskii* is represented below.

BLASTN Sequence similarity 99% ITS for Belgium and Lebanese:

TAGAGGAAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGA
TCATTACTGATTTGAGCCACTATCTACTCCCTCTCATAGTGGTGTTGTCTGATGT
TCTATCTTAGACTCTTACATGCCAGCGTGCTGGCTGTTTGCTGTCTTCGAGAGAC
ATGAAAAACAACCTGTATTAGTTTACGTGGTACGTCTCCTCCGTCCTTTGGCAC
ACCGCTCGATTGTGGTGTTACCGTCCTTTGGTTACGGGAAGGCGTATGCGCGTA
AGTCGGTAACCATTCCGACAACCTTTTCACTTCCTTTGGAAGTAAGTTGTGCTA
TCGAGAAGAAAAAAACCAAGACTCAATATGGGGGATCACTTAGTCCTTAAATC
GATGAAAAACATRGTAATTTGTGGAAATTGATGTGAGTTGTGAAATTTTGTGAG

CATTGTGTTTTTGAATGAAAATTTCAGCACGGACACTTCTGTATCTGTGCTACAT
TTGTTTCAGTATATAAACCGTATCATACGTATTTACCTTTGCTGCAGCCCTCGTC
GGTATCGCCATGCAATGGTATAAATTCTCTTTGGTCACAAGAGTGATACCAAAA
CAAACCATTATGACGTGTATCTGAATCAAGTGTGACGACCCCCTGAATTTAAGC
ATATA

B. Mass rearing of *Amblyseius swirskii*

1. Evaluation of composition of food media for rearing Carpoglyphus lactis

Results of the four performed replicated experiments are reported below

a. Trial 1

Six different media were compared, each containing 20 g of culture medium. The data on the total number of *C. lactis* for each treatment are reported in Figure 16. The highest population reached in week 2 was in the medium composed of Berghol + 20 % yeast. However, at week 3 the *C. lactis* population decreased in the latter treatment but increased considerably in the yeast treatment. The population remained very low on wheat bran or berghol alone. These preliminary results indicate that a medium of yeast or 80% berghol with 20 % yeast is the most suitable for rapid rearing *C. lactis* (Figure 16). However, for longer term production yeast alone may be preferred.

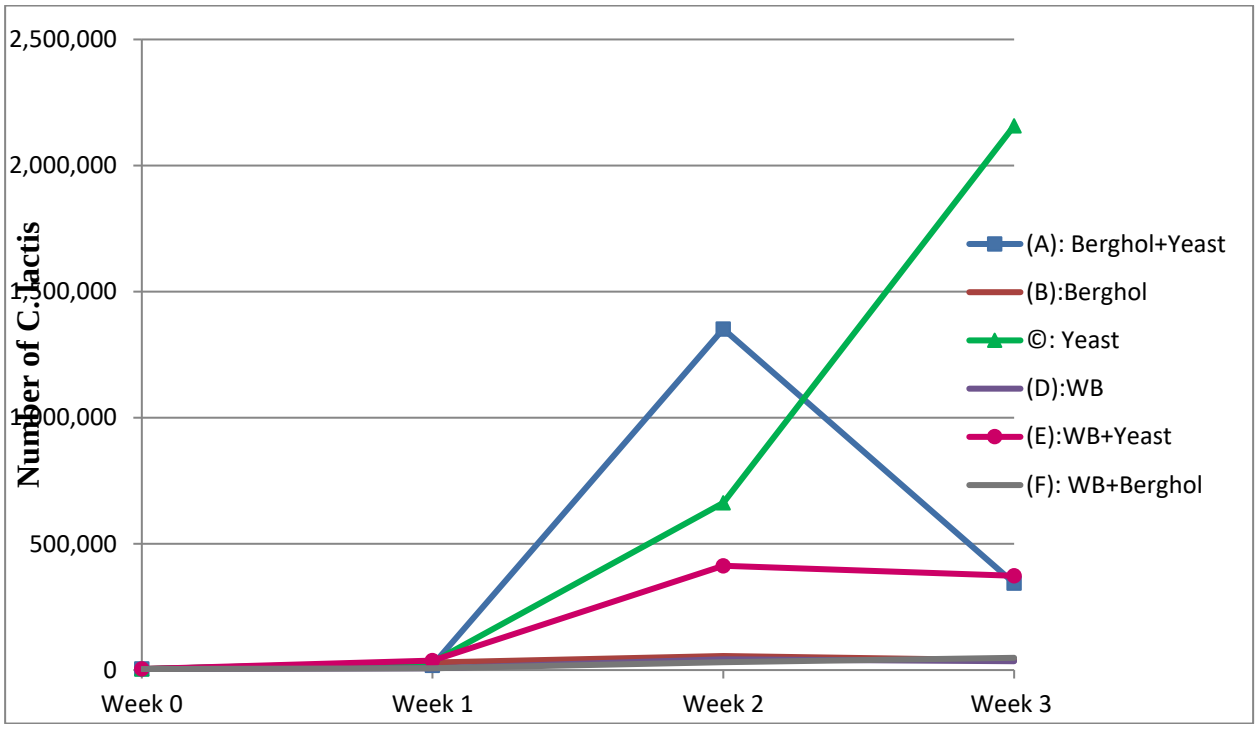


Figure 16. Evolution in the number of *Carpoglyphus lactis* in six rearing media
A: 80% Berghol +20% Yeast B: Berghol
C: Yeast D: Fine Wheat bran
E: 80% Wheat bran +20% Yeast F: 80% Wheat bran + 20% Berghol

b. Trial 2

Seven treatments were evaluated, each containing 20 g of culture medium. The treatment containing Berghol + 30 or 20% yeast (G and A respectively) gave the highest rate of increase of *C. lactis* population, about 1 million, within 3 to 4 weeks, respectively. Then the population dropped at weeks 4 and 5. However, in the treatment which contained yeast, the *C. lactis* population continued increasing during week 5 and reached over 1.4 million by week 6 (Figure 17). These results confirmed the results of the previous experiment.

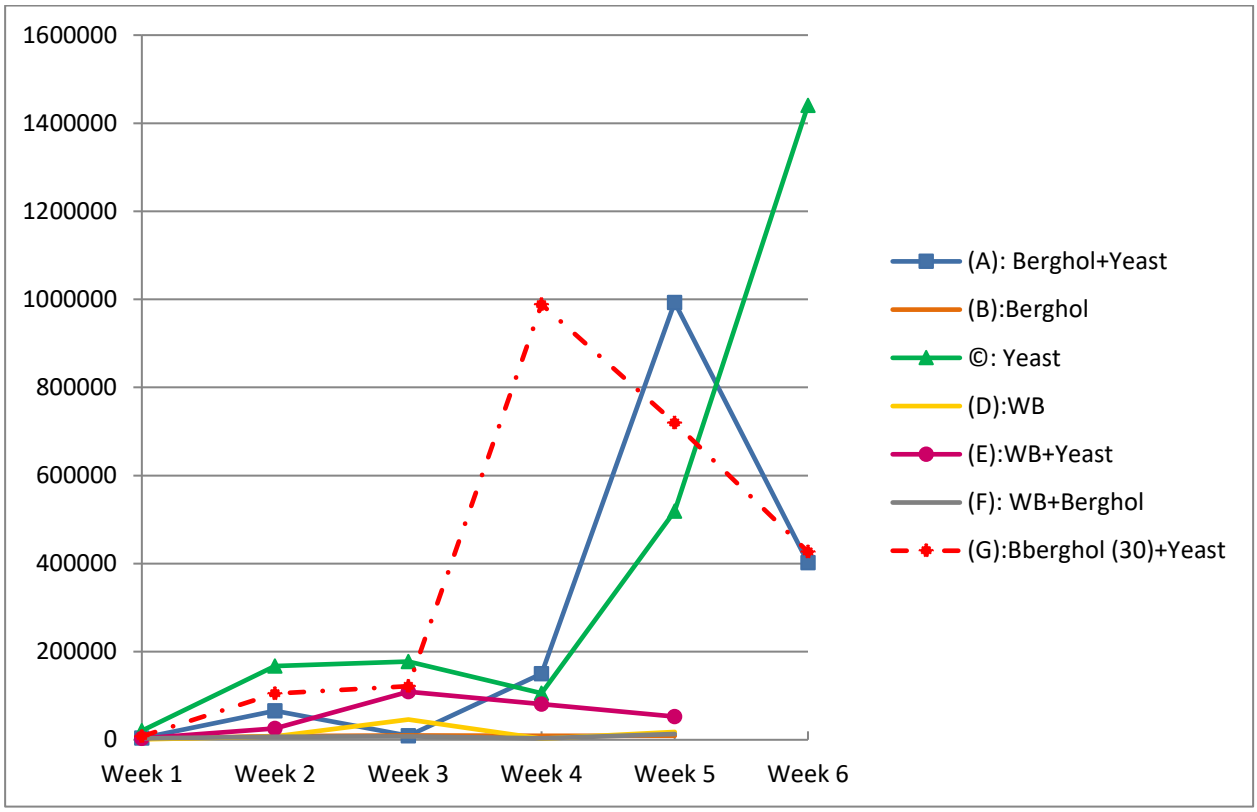


Figure 17. The evolution in the number of *Carpoglyphus lactis* in different combination of culture media.
A: 80% Berghol +20% Yeast B: Berghol
C: Yeast D: Fine Wheat bran
E: 80% Wheat bran +20% Yeast F: 80% Wheat bran + 20% Berghol
G: 70% Wheat bran + 30% Yeast

c. Trial 3

Two treatments of 50 g culture media were tested to check the potential ability of rearing *C. lactis* on a new source of medium size wheat bran (WB) and yeast. The data on the total number of *C. lactis* is reported in Figure 18.The results proved that the wheat bran allows to maintain the *C. lactis* population without significant increase. However, the addition of 15 % yeast allowed significant increase in population to reach 3,000,000 within 3 weeks.

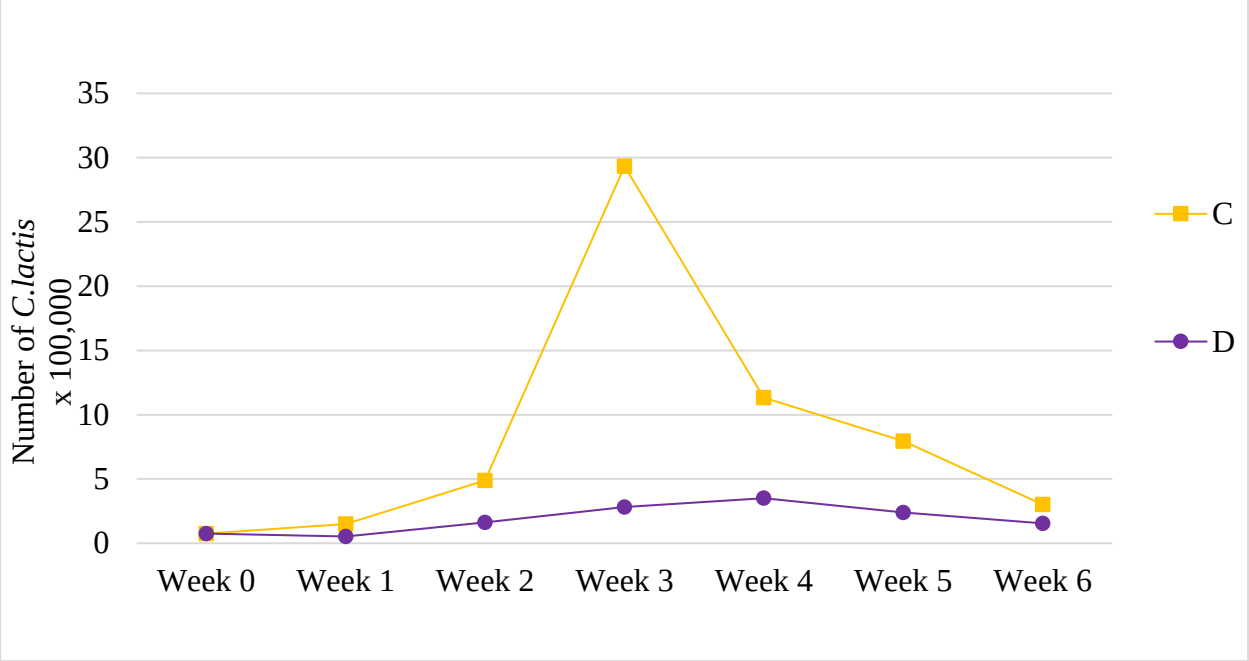


Figure 18. The total number of *Carpoglyhus lactis* in 50 g culture media
C: WB +15% yeast D: wheat bran only

d. Trial 4

Four 50 g culture media were tested to check the suitable media for rearing *C. lactis*. The total number of *C. lactis* present in the four treatments is provided by Figure 19. The results clearly demonstrate that adding 20 % yeast to the coarse wheat bran gave highest population of *C. lactis*, 2 million, within 1 or 2 weeks depending on the initial population. This confirmed the importance of yeast for the rearing *C. lactis*.

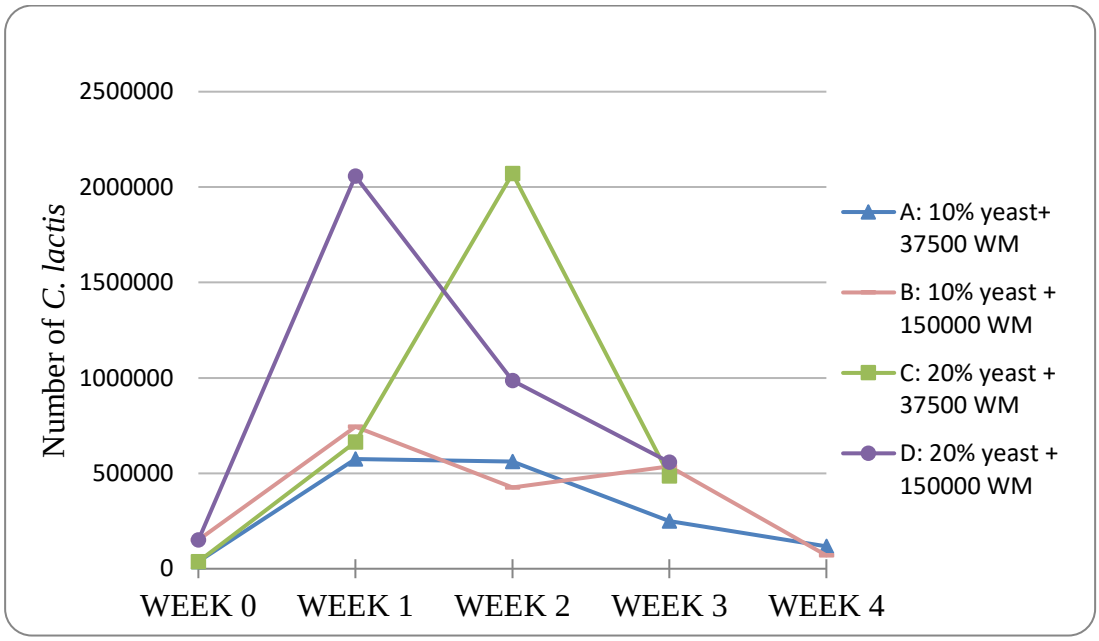


Figure 19. The evolution in the number of *Carpoglyphus lactis* in 50 g culture media
A: 10% yeast + 37,500 C. lactis B: 10% yeast + 150,000 C. lactis
C:20% yeast + 37,500 C. lactis D: 20% yeast + 150,000 C. lactis

Conclusions

For mass production, the wheat mite cannot be reared on wheat bran or berghol alone, the addition of 20% or 30% yeast improves the yield considerably and results in the highest rate of population increase, but the population drops suddenly after reaching the peak production on week 3 or 4. However, yeast alone provided a lower rate of initial increase but the population continued increasing after weeks 3 and 4. Economic analysis shows that coarse wheat bran + 20 or 30% yeast is the least expensive protocol but requires careful monitoring of the population at weekly intervals. The maximum production possible is about 40,000 – 50,000 WM/ g of culture medium. A lag phase of 1-2 weeks was observed in 3 out of the 4 experiments. Conditions that reduce the lag phase, as occurred in experiment 4, would improve the economic returns, and should be further evaluated. No

data are reported in literature for rearing *C. lactis* on such a diet as it is patent for companies that use it for mass production of *A. swirskii.*

2. *Evaluation of composition of food media for rearing Amblyseius swirskii*

Results of 6 replicated experiments are reported below

a. Trial 1

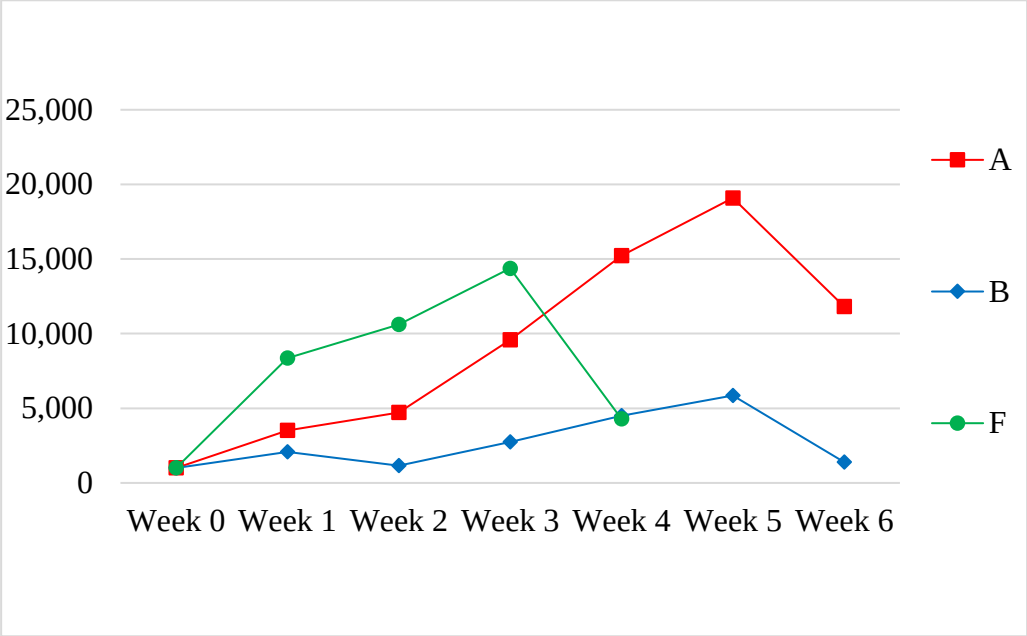


Figure 20. Evolution of the total number of *Amblyseius swirskii* in 50 g mixture
A: 1:75 (SW:CL) +yeast B: 1:100 (SW:CL) (on wheat bran alone)
F: 1:150 (SW:CL) (on wheat bran alone)

Three 50 g culture media were evaluated to determine the best combination to rear *A. swirskii.* The data on the total number of *A. swirskii* in each culture medium over the experimental time is presented in figure 2. The above graph shows that treatment A of 1:75 ratio of *A. swirskii* to *C. lacis* with15 % yeast was most successful in rearing the *A. swirskii* as it allowed significant increase in their population from 1,000 to around 20,000 in 5 weeks. Although treatment F which lacks yeast but contains double the amount of *C. lacis*

compared to treatment A allowed the increase of *A. swirskii* population, the sharp drop down of the *A. swirskii* population from 15,000 in week 3 to 5,000 in week 4 is not sustainable. This sharp decrease in the population is due to absence of feed, *C. lacis,* to be consumed by the high numbers of *A. swirskii* which were attained during week 3 (Figure 20).Thus for continuous supply of high numbers of *A. swirskii* , it is important to add yeast to the culture medium or to supply additional wheat mite at specific intervals. Also, the drop in population of *A. swirskii*, after 6 weeks in A treatment may be correlated with the drop of *C. lactis.*

b. Trial 2

Results of this experiment aiming at evaluating 3 different ratios of SW:CL (1:50, 1:100 and 1:200) and 3 media containing 0, 10 or 20% yeast, for mass production of *A. swirskii*, are presented in Figure 21 toFigure 23.

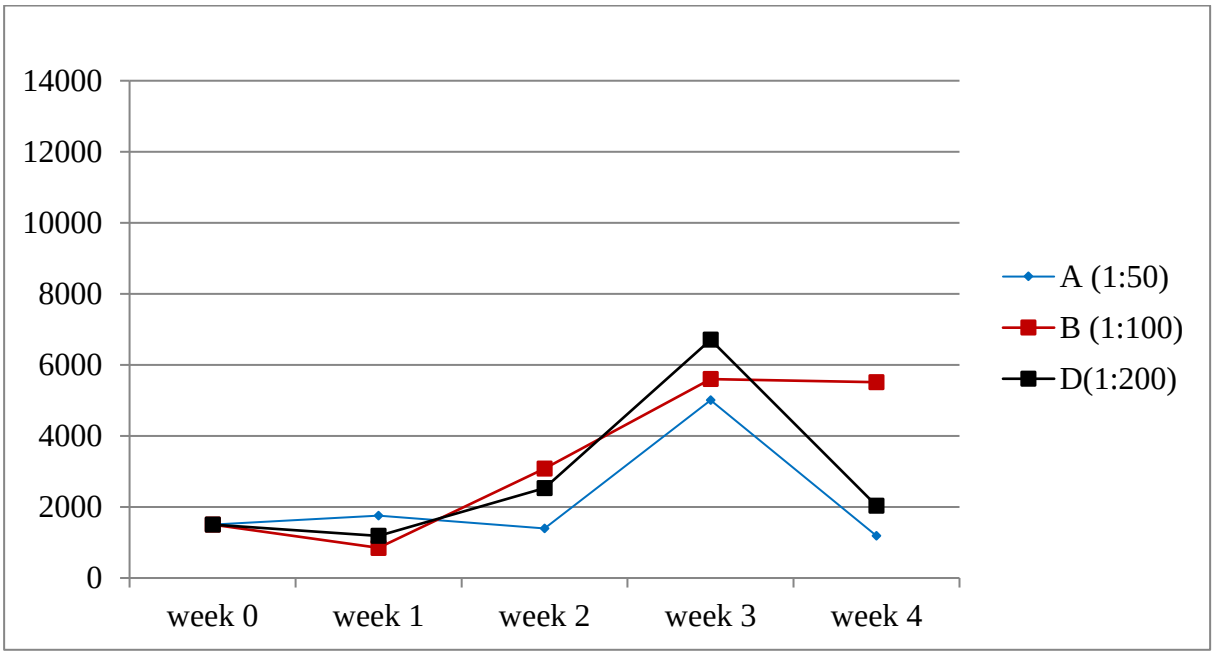


Figure 21. The evolution in the total number of *Amblyseius swirskii* in 0% yeast treatment

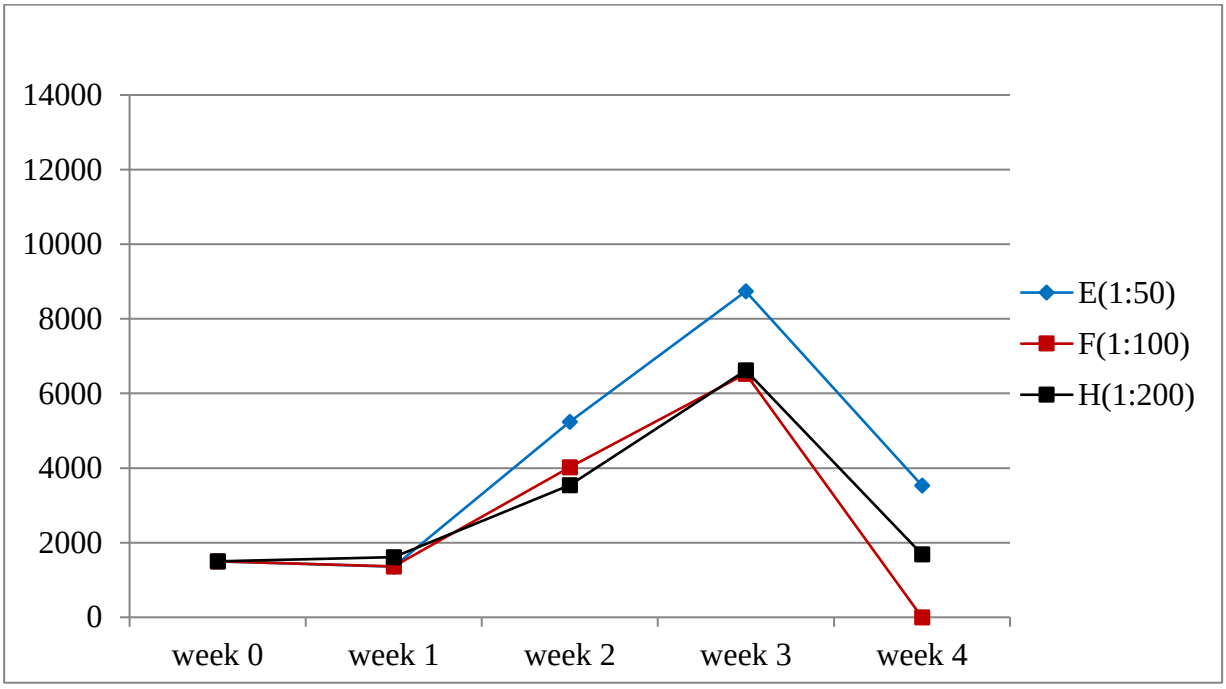


Figure 22. The evolution in the total number of *Amblyseius swirskii* in 10% yeast treatment

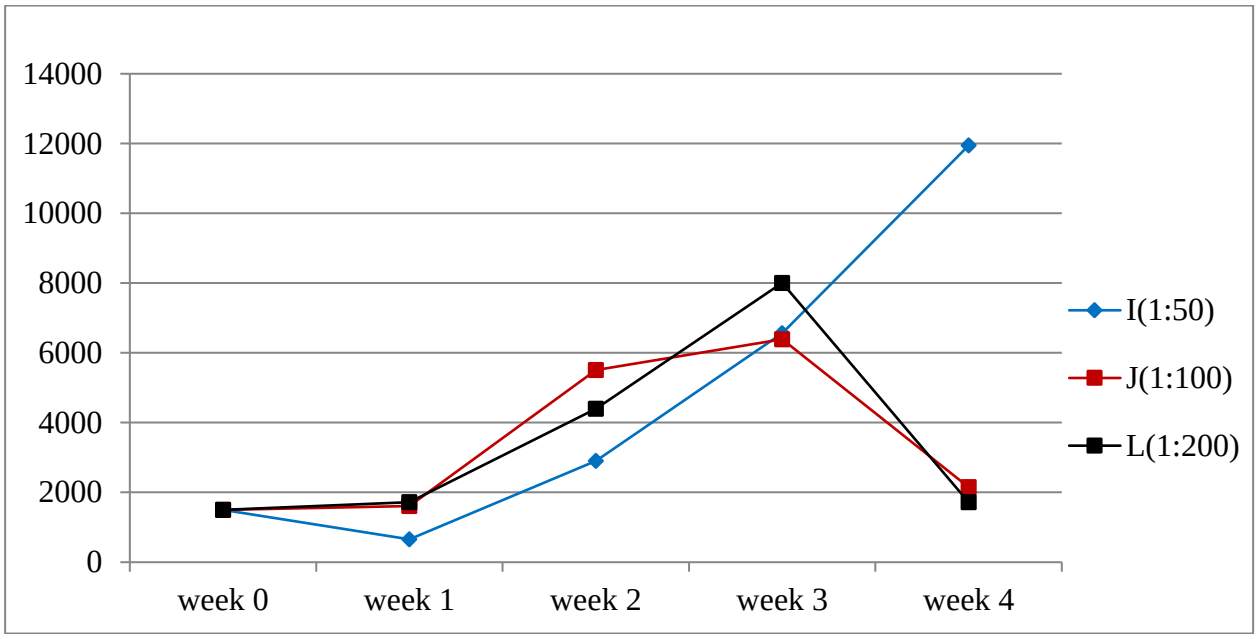


Figure 23. The evolution in the total number of *Amblyseius swirskii* in 20% yeast treatment

Starting with 1500 SW, the evolution in the total number of *A. swirskii* in each treatment each week is provided in Figure 21,Figure 22,Figure 23. Results showed that the population of *A. swirskii* reached its peak at three weeks post introduction (WPI), except for the treatment that contained 20 % yeast and a ratio of 1:50 (*A. swirskii*: *C. lactis*), the population continued to increase till 4 WPI. The latter treatment gave the highest total number of 12,000 *A. swirskii* at 4 WPI. While all the other treatments, gave a total number of SW ranging between 6000 and 8000 per treatment after 3 WPI. It is worth noting that without the addition of yeast to the culture media, the 1:200 SW:CL ratio gave the peak production after 3 weeks with a total of 6700 SW/50 g of culture medium, followed by 1:100 and 1:50 ratios (5600 and 5000, respectively). Thus, the best ratio seems to be 1:50 with 20% yeast since *A. swirskii* feeds on *C .lactis* , and *C .lactis* needs yeast for its

development as it helped in maintaining an extended food source for production of higher population of *A. swirskii*.

c. Trial 3

Four 50 g culture media were tested to check the best media for rearing *A. swirskii*. Starting with 750 SW, the evolution in the number of *A. swirskii* through weeks in the four treatments is reported in Figure 24.The results of experiment 3 confirmed the results of experiment 2 and showed that treatment G with 20% yeast and 1:50 ratio of *A. swirskii* to *C. lactis* was able to maintain the highest average number of *A. swirskii*, 14,890 at 4 WPI. The second-best treatment was H with 20% yeast with a ratio of 1:200 *A. swirskii* to *C. lactis* with a total of 12,690 *A. swirskii*. Both treatments G and H were not significantly different. While in the other two treatments, the peak of production and the highest rate of increase was at 3 WPI with a total number about 10,000 *A. swirskii* , then the population dropped rapidly in the fourth WPI. This may be explained by the drop of the available population of *C. lactis*, at week 3, to lower levels, than those in the two former treatments containing 20% yeast.

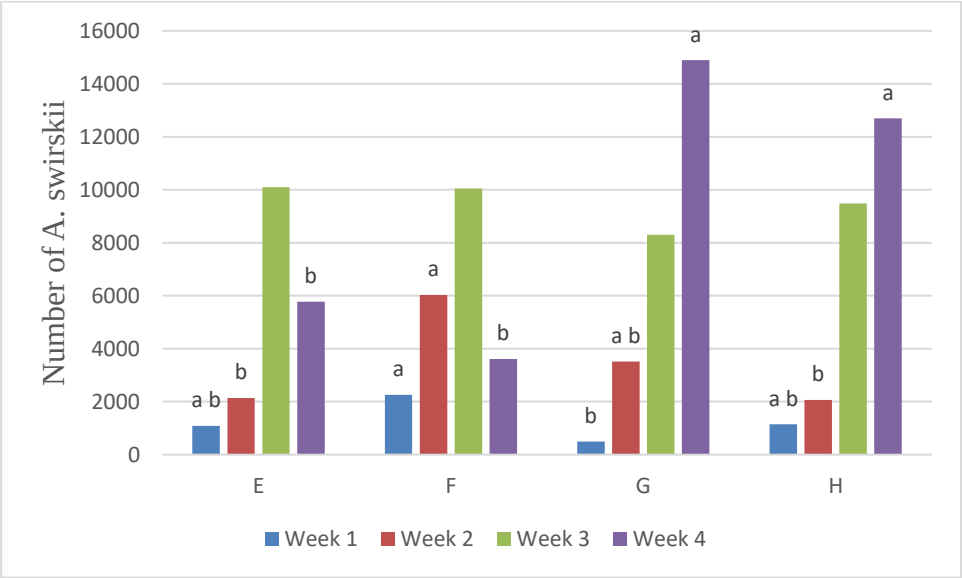


Figure 24. Evolution of *Amblyseius swirskii* population through time in a box of 50 g culture media with different combinations of two yeast ratios (10% and 20%) with two *A. swirskii* ratios (1:50 and 1:200).The treatments of the same weeks with the different alphabetical

E: WB + 1:50 SW:CL + 10% yeast F: WB + 1:200 SW:CL + 10% yeast

G: WB+ 1:50 SW:CL + 20% yeast H: WB + 1:200 SW:CL + 20% yeast

Thus,the previous 4 trials indicated that the best medium for rearing SW is coarse autoclaved wheat bran in addition to 20% yeast with a ratio of 1:50 of SW:CL. The literature lacks data on rearing SW on such diet as it is patent for the companies that mass produce the predatory mite, *A. swirskii.*

d. Trial 4

Two treatments, 2 replicates each, were tested to check the effectiveness of doubling the volume in extending the production cycle of *A. swirskii*. Starting with 300 SW in each treatment, the data of the evolution of *A. swirskii* through weeks in each treatment is reported in Figure 25. Both treatments A and C were successfully able to yield high numbers of *A. swirskii* 3WPI with 1466.92 and 1776.42 respectively with no significant difference between the two treatments. However, at week 4, a drop in the number of *A.*

swirskii in treatment A to 304.98 while it increased for treatment C to yield 2590.76 *A. swirskii* per box. Space limitation might be the reason behind the drop down in the number of *A.swirskii* in treatment A.

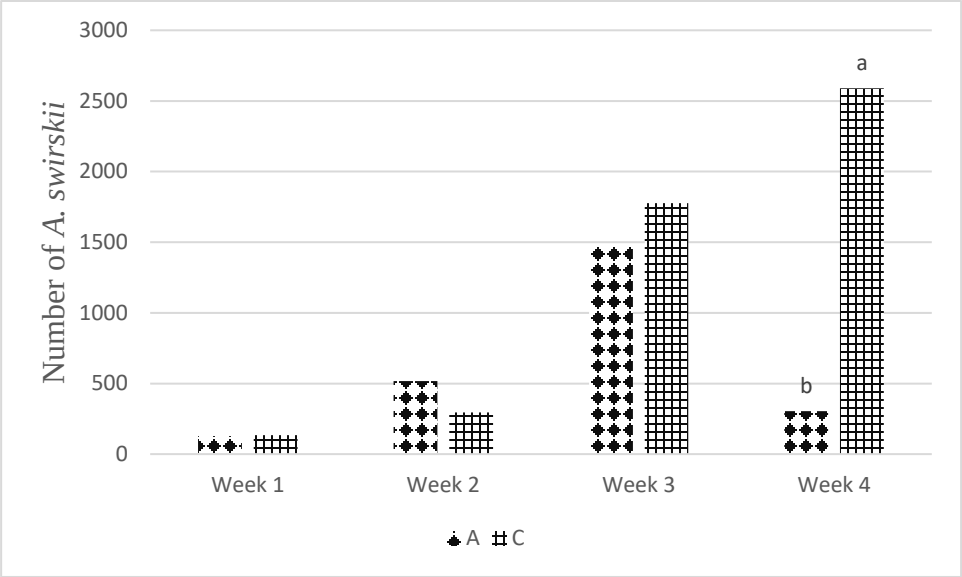


Figure 25. The evolution in the number of *Amblyseius swirskii* in 10 g box of control culture media (A*) and in 20g double spaced culture media (C*). The treatments of the same week with the different alphabetical superscripts are significantly different at P≤0.5
*A: 10g WB + 20% yeast *C: 20g WB + 20% yeast

e. Trial 5

Two treatments with 2 replicates each were evaluated to check whether spraying calcium propionate intended to prevent infection of the culture medium by fungi, will not interfere or affect positively or negatively the increase in the population of *A. swirskii.* Starting with 300 SW, the evolution in the number of *A. swirskii* over time is recorded in Figure 26. Results showed that treatment E which was sprayed with calcium propionate was successful in increasing the population of *A. swirskii* similar to treatment A, the control, which is used to rear *A. swirskii.* Although during week 2, the number of *A. swirskii* was

significantly higher in treatment E (800.43 *A. swirski)* compared to treatment A (516.68 *A. swirskii),* but during the peak in week 3, there was no significant difference between both treatments. Thus, both treatments were successful in rearing *A. swirskii* as calcium propionate didn't affect *A. swirskii* neither positively nor negatively.

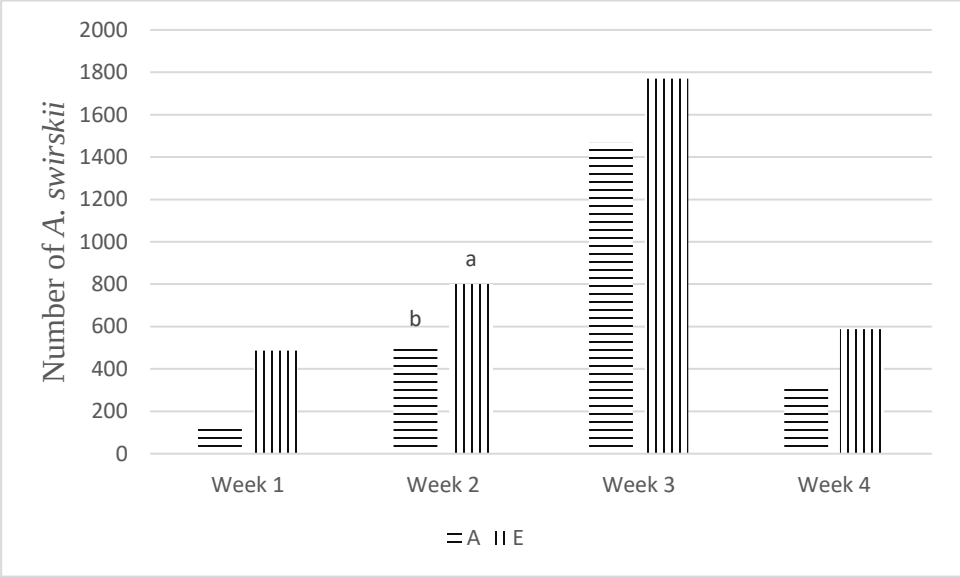


Figure 26. The evolution in the number of *Amblyseius swirskii* in 10 g box of control culture media (A*) and in modified culture media (E*). The treatments of the same weeks with the different alphabetical superscripts are significantly different at P≤0.5

*A: WB + 20% yeast *E: WB + 20% yeast + spraying calcium propionate

f. Trial 6

Two treatments were evaluated each replicated twice to check the effectiveness of sucrose if added to the diet along with spraying of calcium propionate. Starting with 300SW, The evolution of the number of *A. swirskii* in both treatments is represented in Figure 27 indicates that the population of *A. swirskii* was significantly higher during week 4 in treatment C in the presence of sucrose and calcium propionate with 1451.7 *A. swirskii.* This experiment indicates the great effect that sucrose had which helped in maintaining high

numbers of *A. swirskii*. However, it seems that an unknown problem occurred in this experiment where the population increase was hindered in both the control and the treatment with calcium propionate and sucrose. Nonetheless this experiment shows that the addition of sucrose to the medium will improve and extend the production cycle of SW. further experiments are planned to confirm this observation.

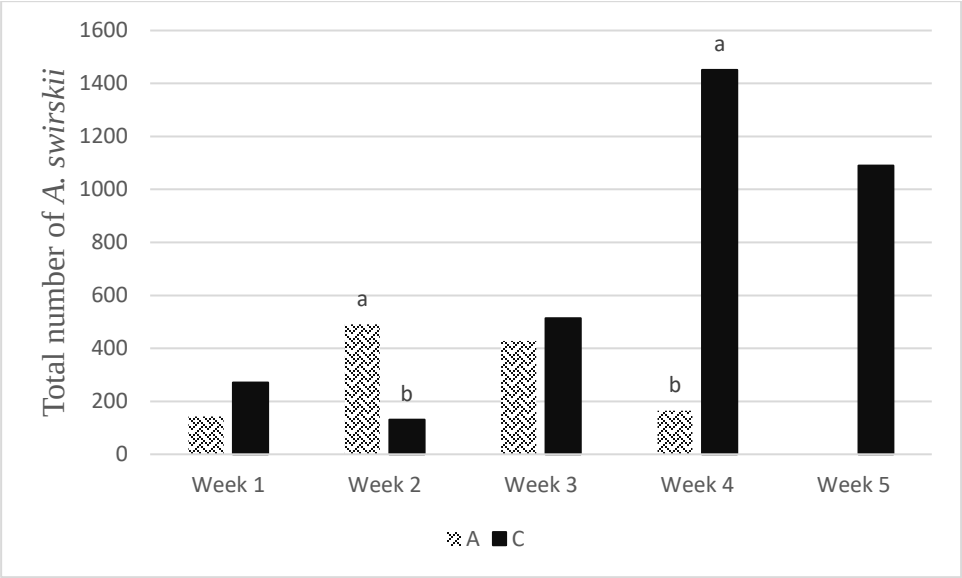


Figure 27. The evolution in the number of *Amblyseius swirskii* in 10 g box of control culture media (A*) and in modified culture media (C*). The treatments of the same weeks with the different alphabetical superscripts are significantly different at P≤0.5

*A: WB + 20% yeast *C: WB + 20% yeast + sucrose+ spraying calcium propionate

C. Small scale experiment for evaluating the Efficacy of local *Amblyseius swirskii* in controlling whiteflies

Results of the small-scale experiment conducted to test the efficacy of the locally reared *A. swirskii* against *B. tabaci* are presented in Figure 28 and Figure 29. The two release rates were both effective in controlling the whitefly eggs as they were significantly different in weeks 3, 4, 5 and 6 as compared to the control, with a proven efficacy of over 90%

reduction in the number of eggs till week 5. However, the reduction in the number of eggs dropped to 73% at week 6, this might be due to the release of 30 adult whiteflies during week 4 which led to an increase in the number of eggs and *A. swirskii* did not get enough time to consume the recently laid eggs (Figure 28).

A. swiskii was also effective in reducing the number of whitefly nymphs, whereby by week 5, the high release rate reduced nymphs by 93% and the low release rate by 84.5%. The number of nymphs in the control dropped in week 6 because the adults had emerged (Figure 29).

Thus, this small-scale experiment proved that the local strain of *A. swirskii* which was reared in the lab on the factitious prey, *C. lactis*, was effective in controlling whiteflies. It was more effective in controlling the eggs than the nymphs. Since both release rates, 50 or 100 SW/m^2 were not significantly different, it is recommended to use 50 SW/m^2. The results of the above trials are in agreement with the results reported by Calvo et al. (2011) who stated that even 75 *A. swirskii* /m^2 should be an adequate rate for controlling whitefly population in cucumber greenhouse.

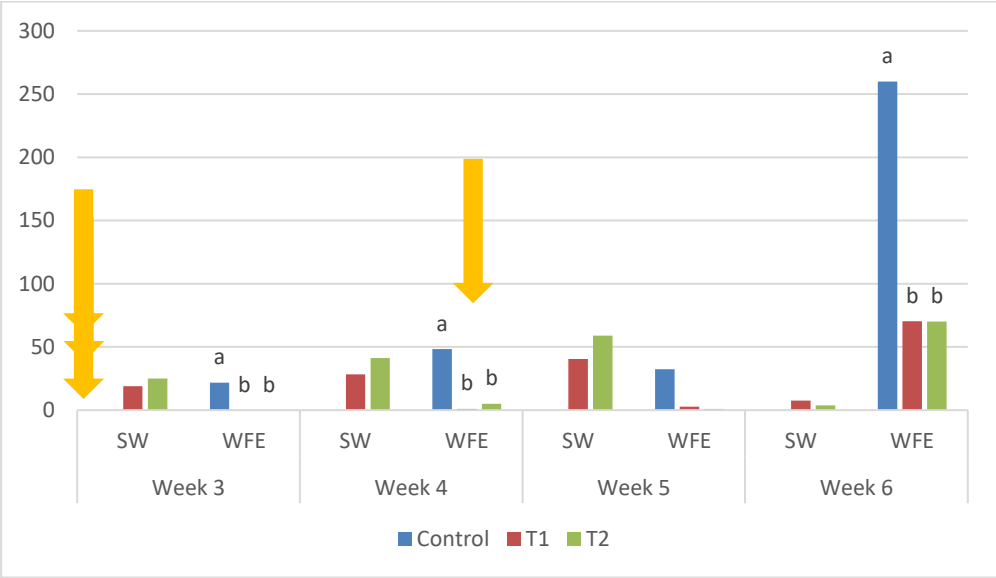


Figure 28. The average number of *Amblyseius swirskii* vs whitefly eggs on 6 leaves (2 cucumber plants) in a cage of 1m².
The orange arrow represent the release of whitefly adults, two released of 15 whitefly adults week 0 and 1 and two releases of 30 whitefly adults week 2 and 4.
The average of whitefly eggs of the same week for different treatments with different alphabetical superscripts are significantly different with $p<0.05$.

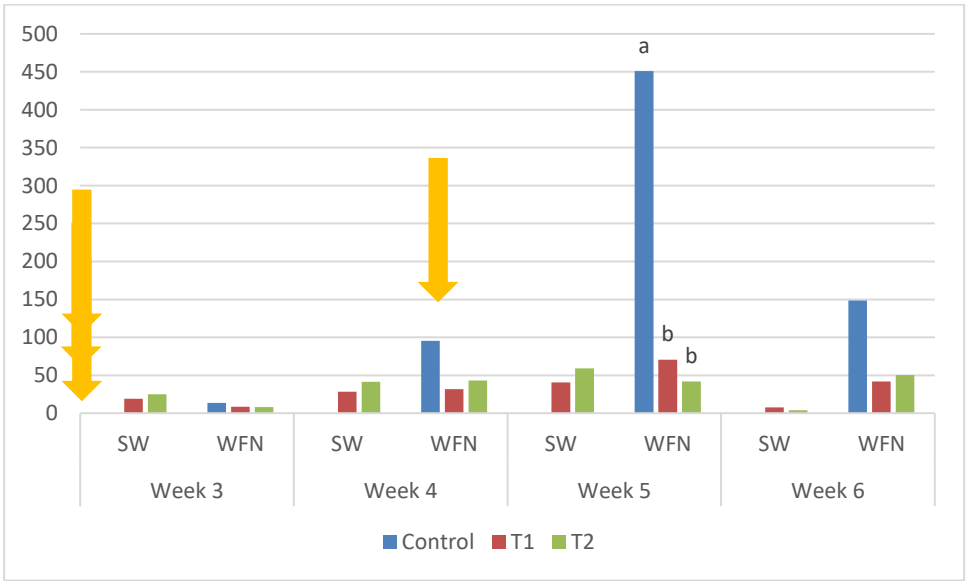


Figure 29. The average number of *Amblyseius swirskii* and the number of whitefly nymphs on 6 leaves (2 cucumber plants) in a cage of 1m².
The orange arrow represent the release of whitefly adults, two released of 15 whitefly adults week 0 and 1 and two releases of 30 whitefly adults week 2 and 4.

The average of whitefly nymphs at the same week for different treatments with different alphabetical superscripts are significantly different with $p<0.05$.

D. Large scale greenhouse experiment for evaluation of the efficacy of *A. swirskii* for the management of two greenhouse cucumber pests: whiteflies and thrips in Kfarmashoun/Caza of Byblos

1. Temperature and RH variations

The temperature and relative humidity recorded by the data logger set in the IPM greenhouse in Kfarmashoun area are presented in Figure 30. Considerable variations in the average temperature occurred during March and April, with average temperature dropping to below 15 ºC in some days and reaching 25 ºC in others. During May and June, the average temperature varied between 20 and 30 ºC. The relative humidity fluctuated between 50 and 90%.

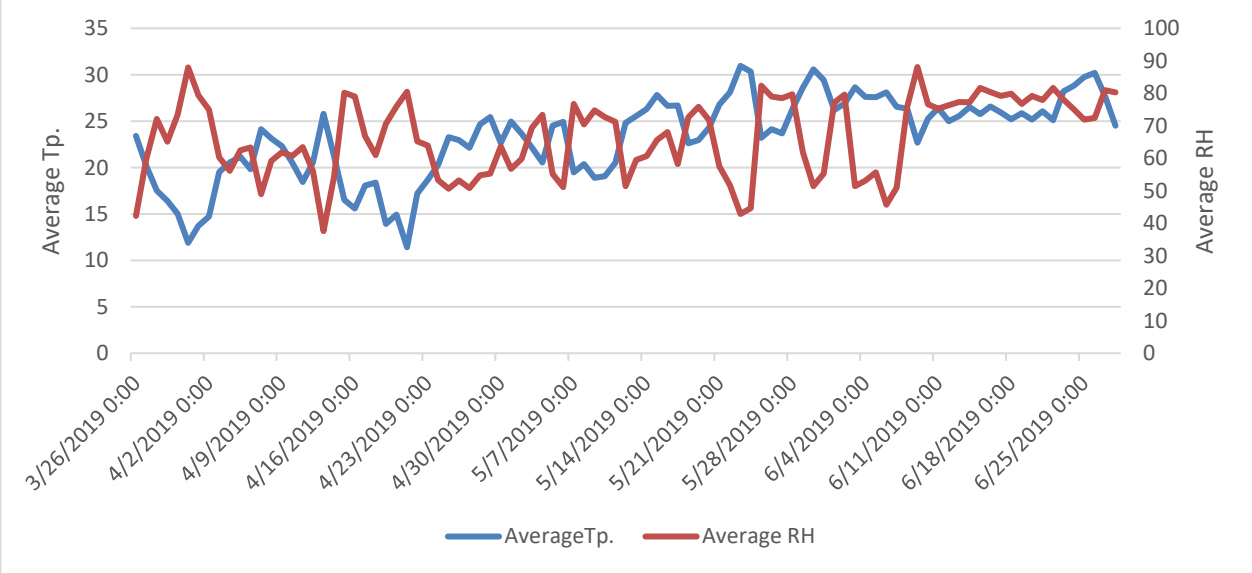


Figure 30. The average temperature and relative humidity in the IPM greenhouse.

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

In the control greenhouse, the farmer relied heavily on insecticides/acaricides sprays to control the arthropod pests. He also claimed that he got some of the pesticides banned in Lebanon as most of the pesticides present in Lebanon are no longer effective in controlling specifically thrips. With 14 sprays of mixtures of different active ingredients during a growing season of 13 weeks, the farmer was able to maintain the population of thrips adults and larvae, whitefly adults and nymphs below their ETLs, but at high environmental and economic costs due to the frequent pesticide applications.

In the IPM greenhouse, seven *A. swirskii* releases were conducted to suppress the population of thrips and whiteflies. The first release was a preventive measure at 35 *A. swirskii*/m² released mainly on the marigolds (habitat plants to trap insects) and the pepper plants which the farmer kept, from the previous growing season, on the border of the greenhouse. This practice was followed because the experiment was started two weeks after transplanting when the farmer had already applied two sprays of pesticide mixtures and the pest populations on the cucumber seedlings were null and the seedlings might have pesticides residues on the leaves. Only two curative releases of around 60 *A. swirskii*/m² were done over the entire greenhouse. The remaining four releases were done on hotspots, where the pest population was high compared to the predator.

During the experiment, 3 sprays of the fungicide chlorthalonil, were applied as a preventive measure against powdery and downy mildews. Chlorothalonil seemed to have minimal negative effect on *A. swirskii*.

The population dynamics of thrips adults and larvae, whiteflies adult and nymph along with their natural enemy, *A. swirskii* in the control and IPM greenhouses, are presented in Figure 31 to Figure 34.

Since the greenhouse contained two crops, cucumber and pepper, data will be reported for each crop separately.

a. Cucumber

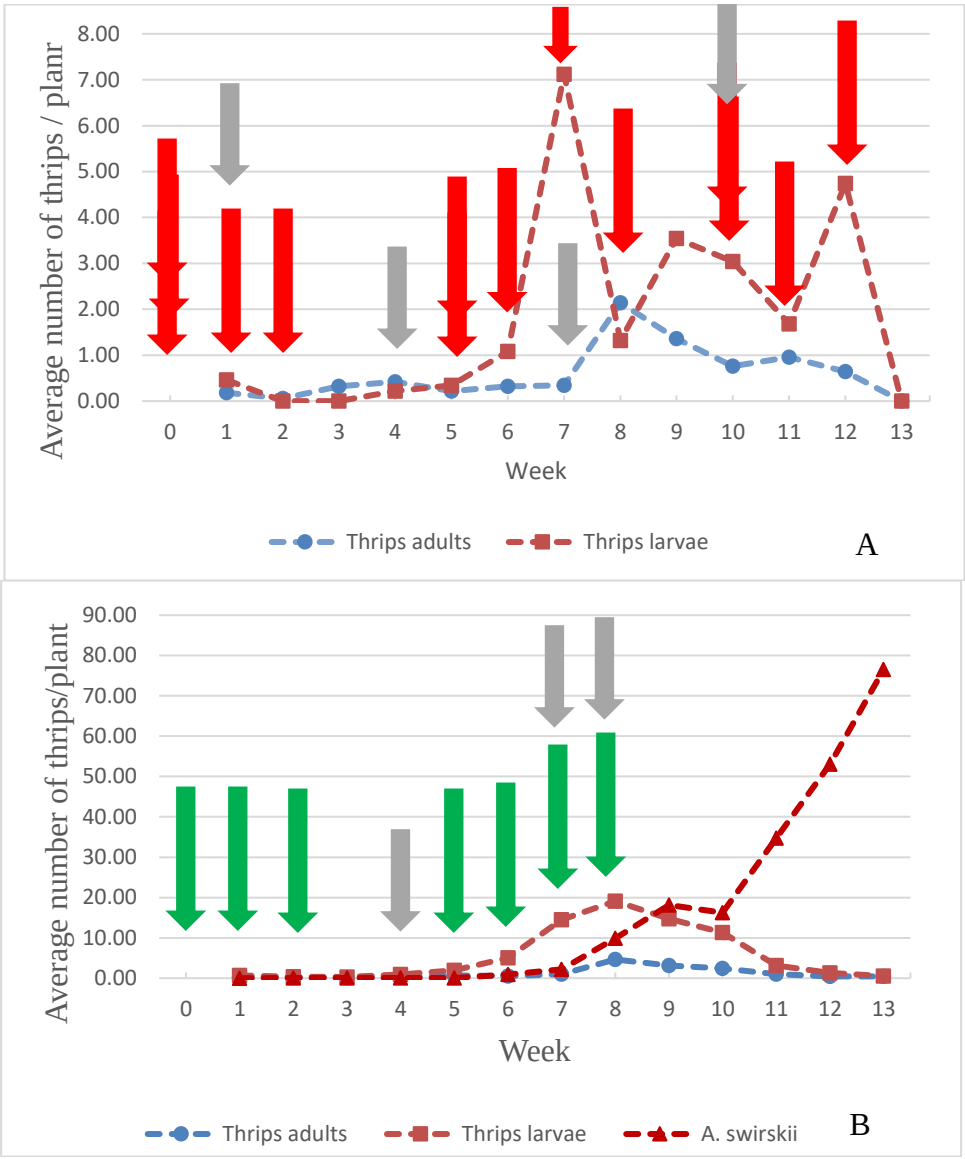


Figure 31. Evolution of the average number of thrips adults and larvae per three leaves on cucumber plants in the control greenhouse (A) and the IPM greenhouse (B).
Red arrows represent the spraying date of insecticides/acaricides, green arrow for release dates of *A. swirskii* while the grey for fungicide applications.

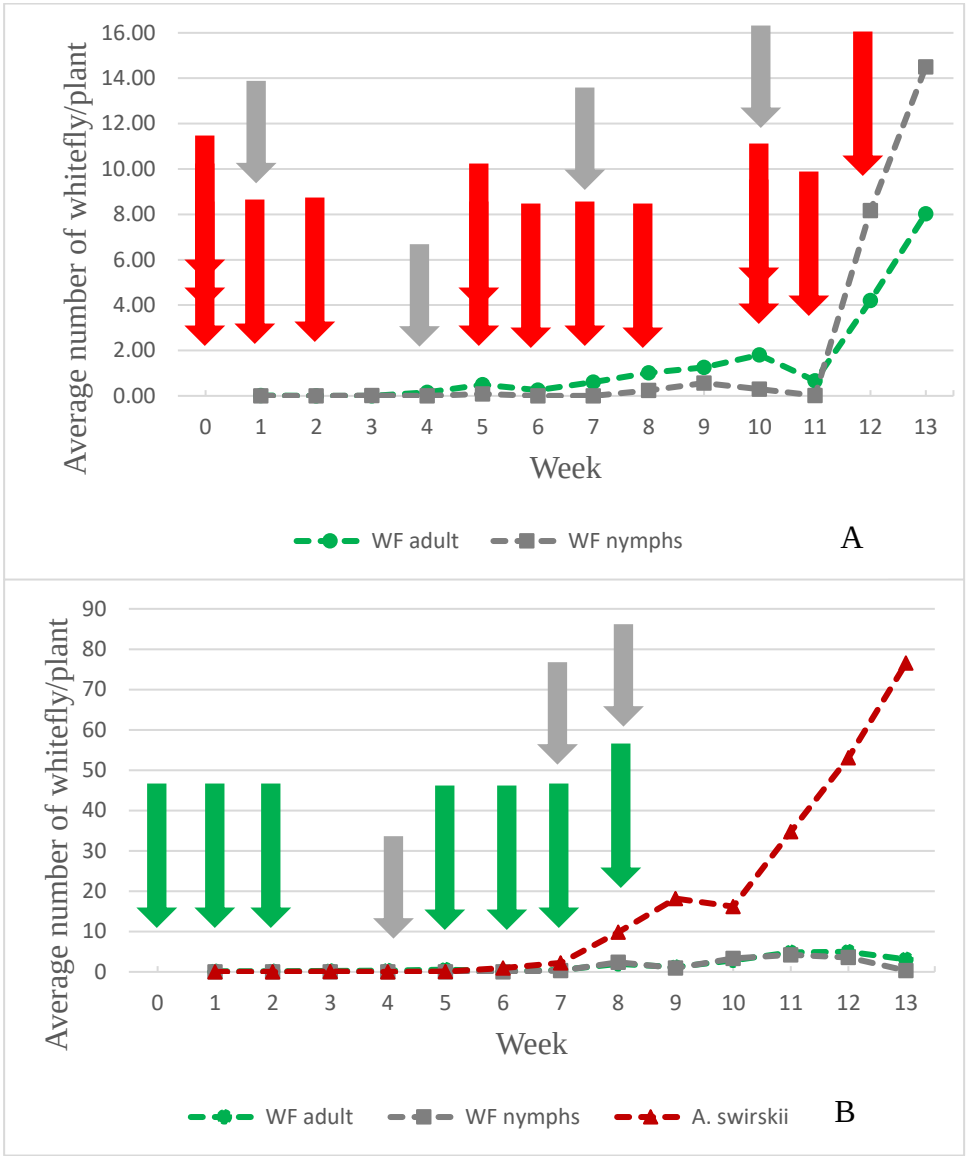


Figure 32. Evolution of the average number of whitefly adults and nymphs per three leaves on cucumber plants in the control greenhouse (A) and the IPM greenhouse. (B).
Red arrows represent the spraying dates of insecticides/acaricides, green arrows for release dates of *A. swirskii* while the grey for fungicide applications.

b. Pepper

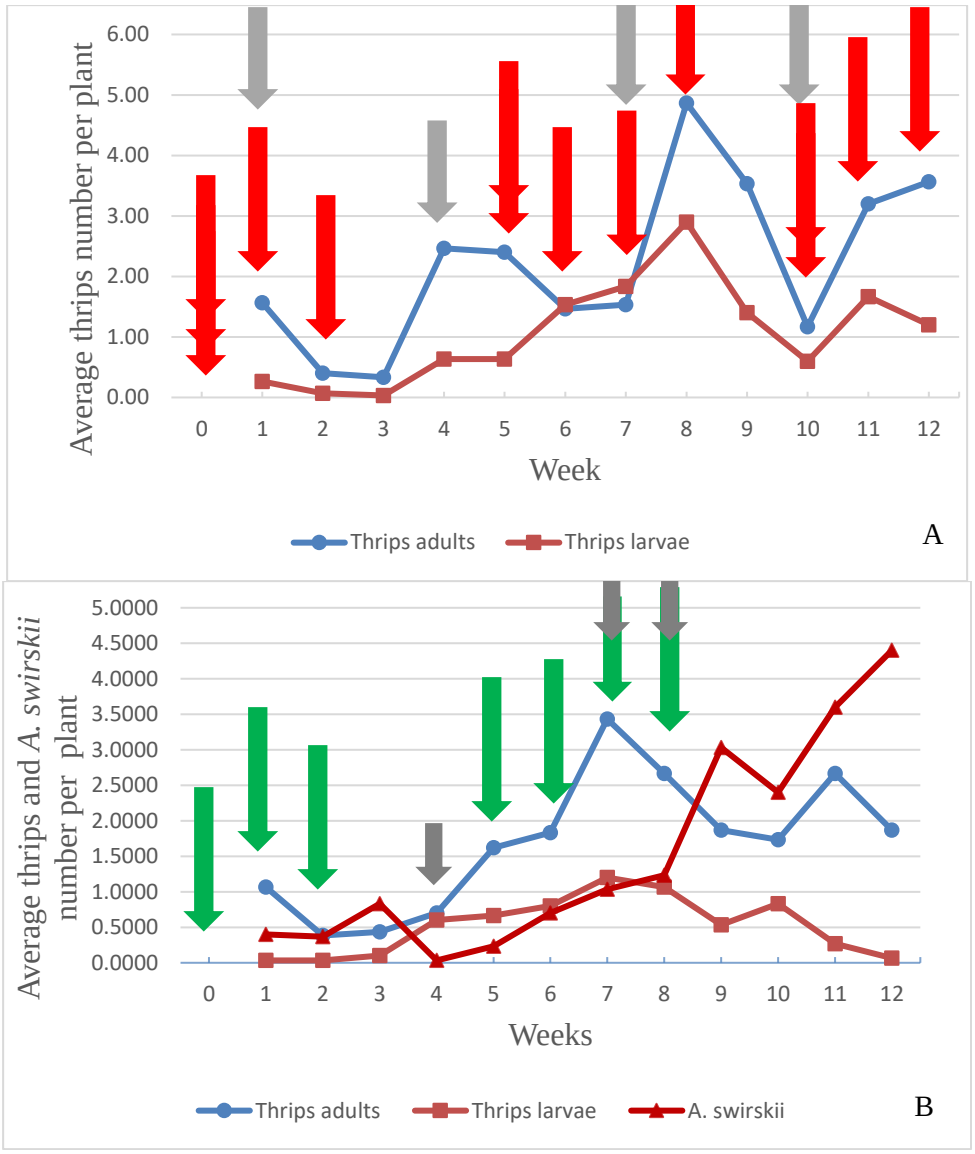


Figure 33. Evolution of the average number of thrips adults and larvae per three leaves on pepper plants in the control greenhouse (A) and the IPM greenhouse (B).
Red arrows represent the spraying dates of insecticides/acaricides, green arrows for release dates of *A. swirskii* while the grey for fungicide applications.

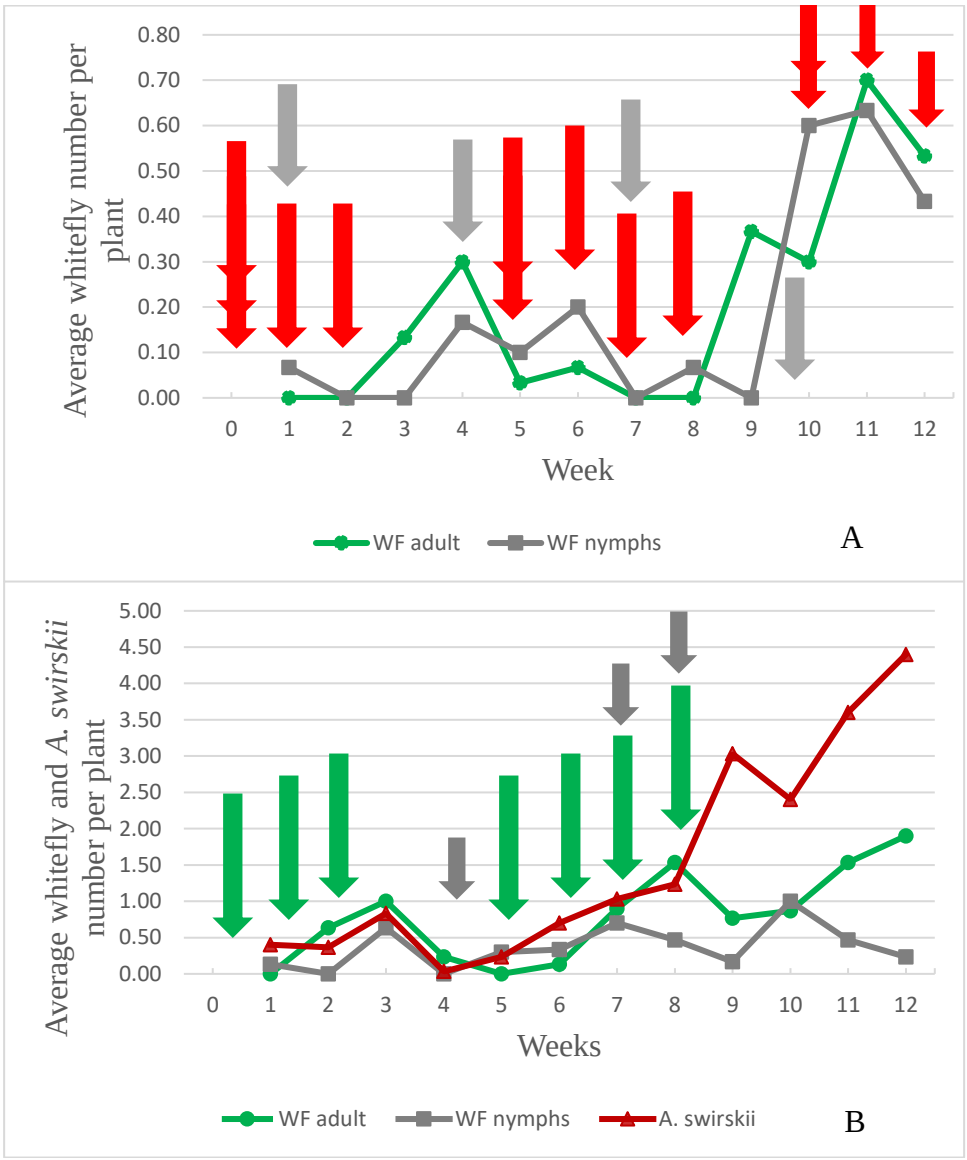


Figure 34. Evolution of the average number of whitefly adults and nymphs on pepperplants in the control greenhouse (A) and the IPM greenhouse (B).
Red arrows represent the spraying dates of insecticides/acaricides, green arrows for release dates of *A. swirskii* while the grey for fungicide applications.

The average number of whiteflies and thrips in the control and IPM greenhouses for cucumber and pepper greenhouses are represented in figures Figure 35 to Figure 42.

For cucumber:

Thrips adults were significantly higher in the IPM greenhouse during weeks 8, 9,10 and 13 compared to the control greenhouse. However, these differences are of minimal importance since the average number of thrips adults per leaf were still lower than the ETL during all the production season (Figure 35). Similarly, the highest average number of adult thrips per 3 leaves (per plant) in the IPM greenhouse was reached during week 8 with 4.64 ± 0.63adult thrips for three leaves thus 1.54 for one leaf which is less than 1.7, the economic threshold level for thrips adults per leaf .

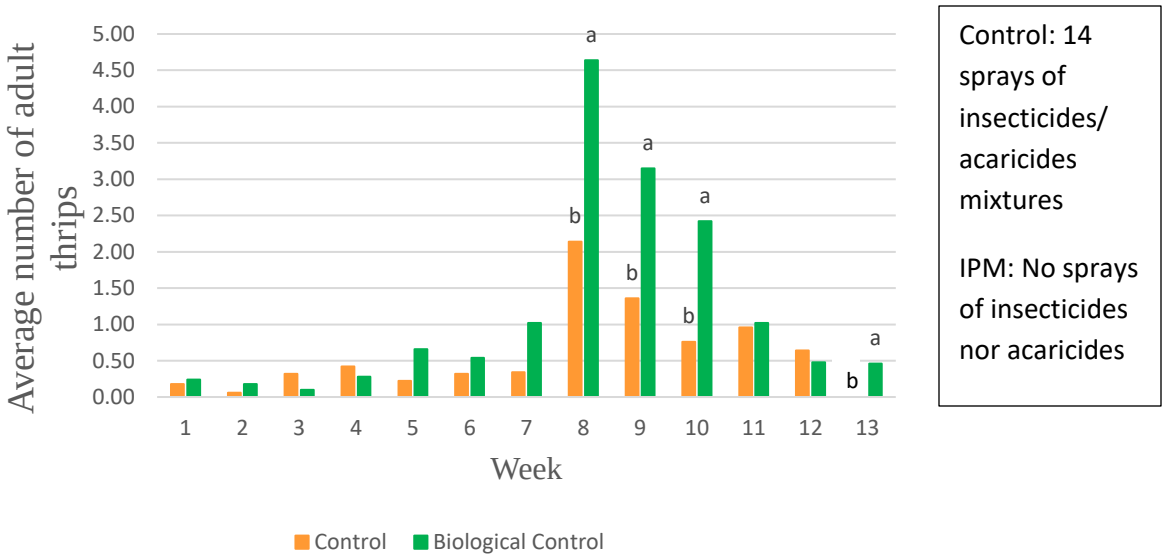


Figure 35. Comparison of the average number of adult thrips per three leaves of cucumber plant in control and IPM greenhouses.
Different treatments at the same week with different superscripts are significantly different with $p<0.05$.

Larvae thrips were significantly lower in the control compared with the IPM greenhouse almost during all the production season (Figure 36). However, the maximum average number of larvae thrips per three leaves was reached during week 8 with 19.10 ± 1.796thus 6.36 thrips larvae per leaf, a number that was lower than 9.5, the ETL of thrips larvae (Shipp et. al, 2000). Bolckmans et al. (2005) reported that *A. swirskii* releases at 75 /m² are adequate for controlling thrips population in cucumber greenhouses.

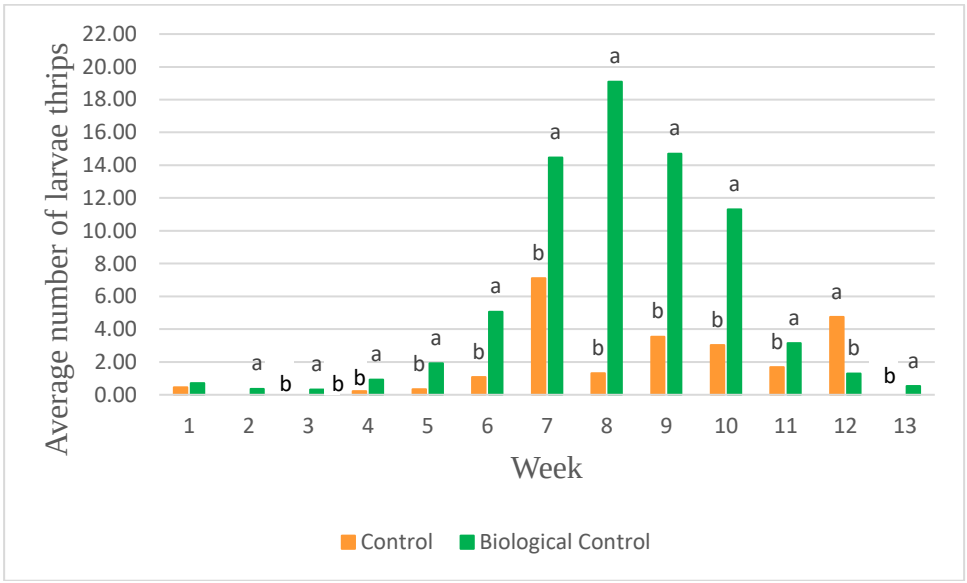


Figure 36. Comparison of the average number of larvae thrips per three leaves of cucumber plant in control and IPM greenhouse.
Different treatments of the same week with different superscripts are significantly different with $p<0.05$.

Both greenhouses had similar average number of whitefly adults during the growing season, except at weeks 11 and 13. At week 11 the average number of adult whiteflies were significantly higher in the IPM greenhouse with 4.86 ± 0.97 per three leaves (Figure 37), thus 1.62 per leaf . Also at week 13 where the average number of adult whiteflies was

significantly higher in the control with 8.02 ± 0.96, thus 2.67 per leaf. However, both values were lower than 5, the ETL for whitefly adults.

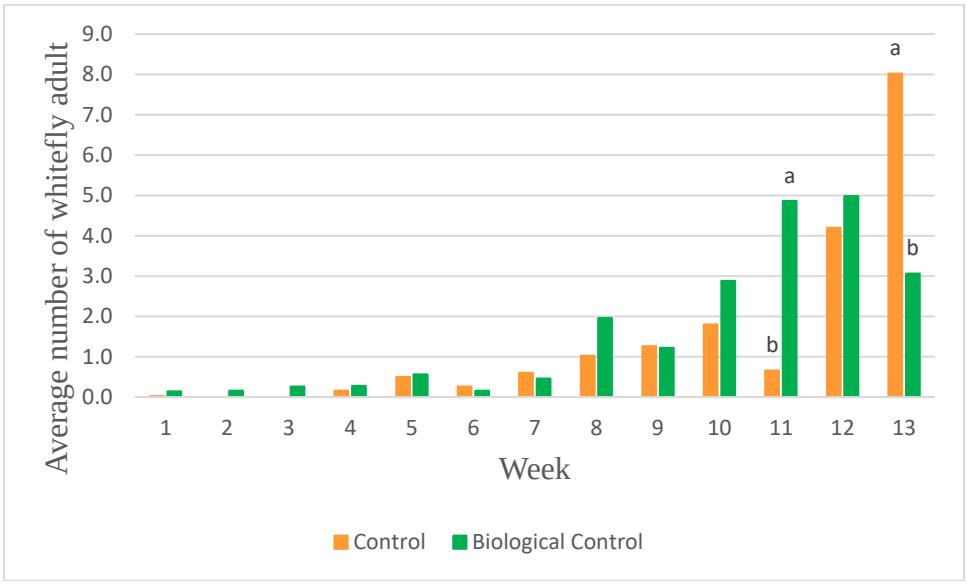


Figure 37. Comparison of the average number of whitefly adults per three leaves of cucumber plant in control and IPM greenhouses.
Different treatments of the same week with different superscripts are significantly different with $p<0.05$.

The average number of whitefly nymphs in the IPM greenhouse were significantly higher than the control greenhouse during weeks 8, 10 and 11 with 2.38 ± 0.50, 3.32 ± 0.54 and 4.22 ± 0.85 whitefly nymphs per plant (3 leaves) (Figure 38), thus 0.79, 1.10 and 1.40 whitefly nymphs per leaf, respectively , lower than 5, the ETL of whitefly nymphs. Although the average number of whitefly nymphs was significantly higher in the control during week 13 with 14.50 ± 0.30 per plant (3 leaves), 4.83 per leaf, but the average number per leaf is still less than the ETL. Bolckmans et al. (2005) reported that *A. swirskii* releases at a rate of 75 /m² s are adequate for controlling whitefly population in cucumber greenhouses.

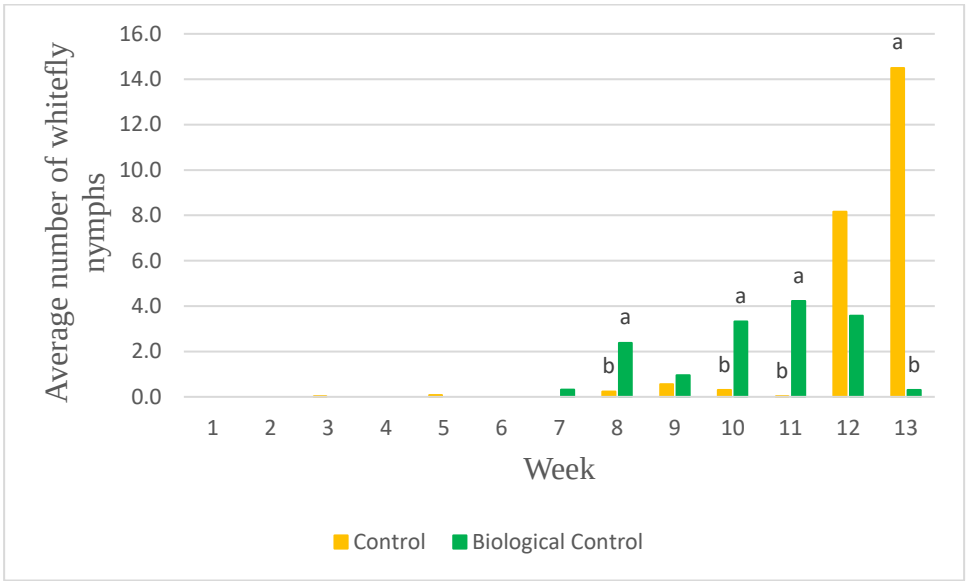


Figure 38. Comparison of the average number of whitefly nymphs per three leaves of cucumber plant in control and IPM greenhouse.
Different treatments at the same week with different superscripts are significantly different with $p<0.05$.

For pepper:

The average number of adult thrips were higher in the control compared to the IPM greenhouse. However, the maximum average number of adult thrips on pepper plants was recorded at week 8 with 4.86 ± 0.57 per 3 leaves, thus 1.62 per 1 leaf which is lower than 1.7, the ETL for adult thrips (Figure 39).

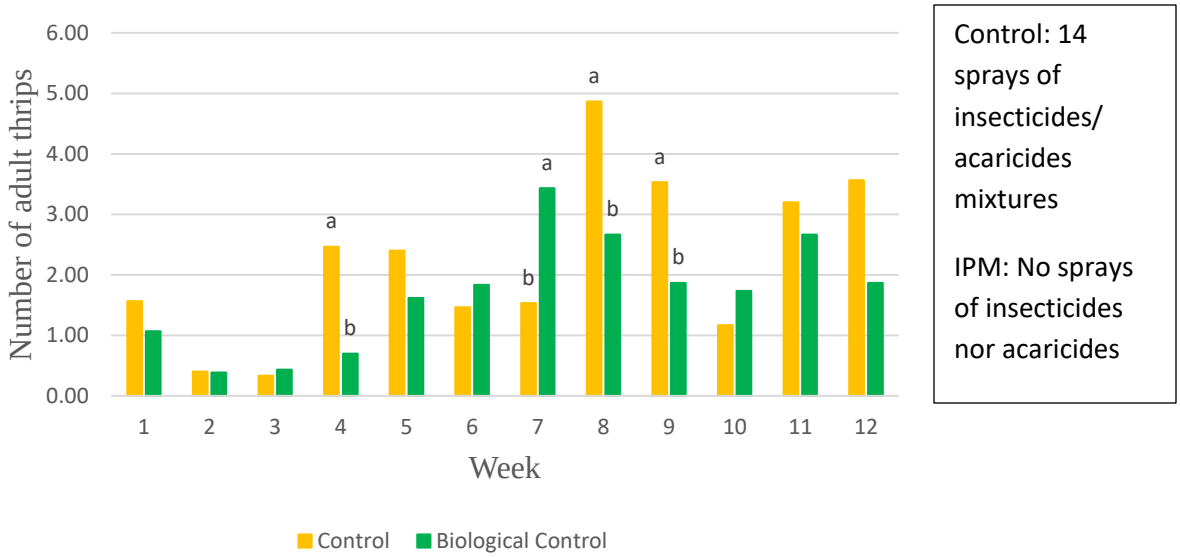
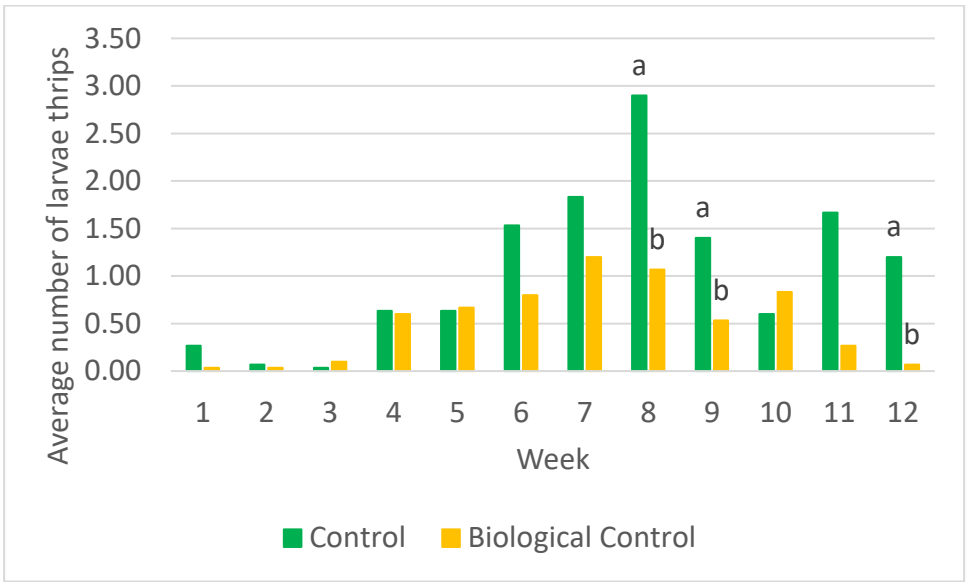


Figure 39. Comparison of the average number of adult thrips per three leaves of pepper plant in control and IPM greenhouse.
Different treatments of the same week with different superscripts are significantly different with $p<0.05$.

The average numbers of larvae thrips per plant in the control greenhouse were significantly higher than the average number in the IPM greenhouse (Figure 40), but the maximum average was recorded in week 8 with 2.90 ± 0.41 per three leaves thus 0.96 per leaf which is still lower than the ETL. These observations agree with Arthurs et al. (2009) where *A. swirskii* was the effective predator in maintaining thrips below 1 per terminal pepper leaf.

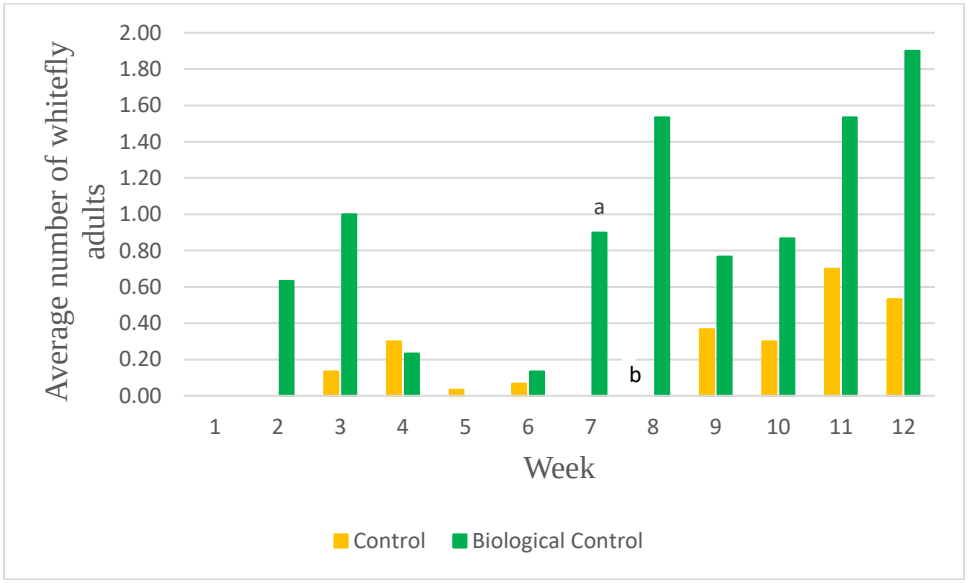


Control: 14 sprays of insecticides/ acaricides mixtures

IPM: No sprays of insecticides nor acaricides

Figure 40. Comparison of the average number of larvae thrips per three leaves of pepper plant in control and IPM greenhouses.
Different treatments of the same week with different superscripts are significantly different with $p<0.05$.

Whitefly adults were higher in the IPM greenhouse with a maximum average of 1.90 ± 0.55 per three leaves (Figure 41), thus 0.63 per leaf which is lower than the ETL.



Control: 14 sprays of insecticides/ acaricides mixtures

IPM: No sprays of insecticides nor acaricides

Figure 41. Comparison of the average number of whitefly adults per three leaves of pepper plant in control and IPM greenhouses.
Different treatments of the same week with different superscripts are significantly different with $p<0.05$.

The average number of whitefly nymphs were frequently higher in the IPM greenhouse but with no significant difference with their number in the control greenhouse (Figure 42). The maximum average number of whitefly nymphs was recorded during week 10 with 1.00 ± 0.26 nymphs per three leaves thus 0.33 per 1 leaf which is still lower than the ETL. These results are in agreement with those reported by Calvo et al. (2012) who mentioned that *A. swirskii* provided significant reduction of the whitefly population and pest control costs in pepper greenhouses.

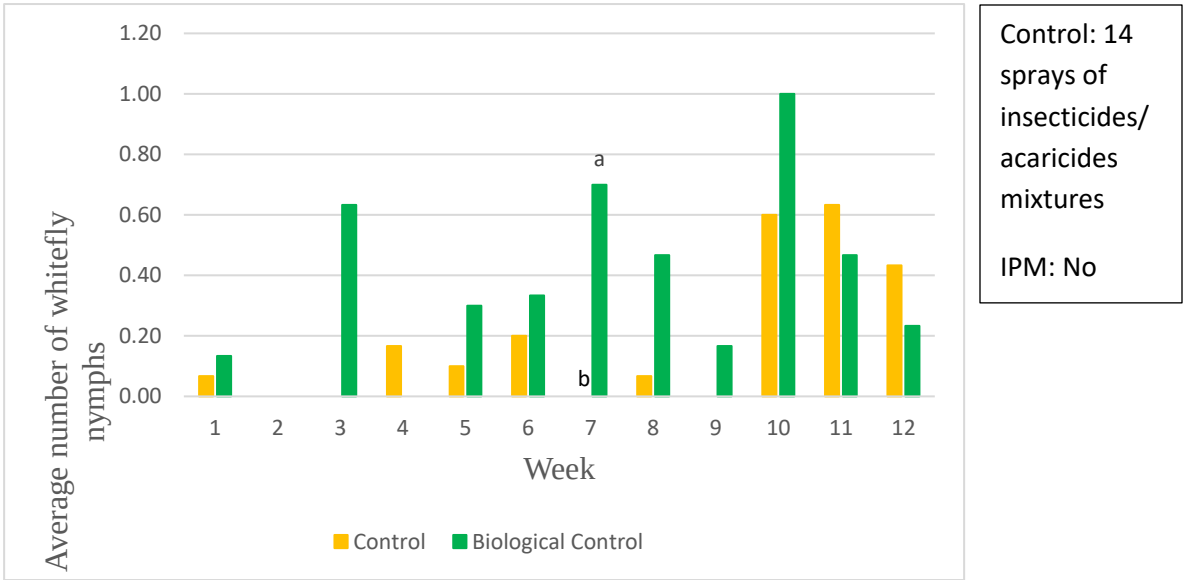


Figure 42. Comparison of the average number of whitefly nymphs per three leaves of pepper plant in control and IPM greenhouses.
Different treatments of the same week with different superscripts are significantly different with $p<0.05$.

3. Cost analysis

Table 4. Cost analysis for three biological control agents

Natural enemies	*A. swirskii*	*P. persimilis*	Beauveria	Total cost
Total numbers used per season in 450 m²	107,000	30,000	10 L (10^8 spore m-1)	
Cost per unit in country of origin ($)	207	171	77	455
Cost per 450 m² per season ($)	443	513	2	958
Cost if local production 50% ($)	221.5	256.5	1	479
Cost if local production 40% ($)	177.2	205.2	0.8	383.2
Material cost for local production per unit ($)	Container 1$ Wheat Bran (100g) 0.1$ Yeast (20g) 0.2$ Vaseline 0.5$ 0.9$ for C. lactis Sum= 2.7$	Seeds 12$ Pots 8$ Fertilizers &fungicides 5$ Vermiculite 0.5$ Net 3$ Bottle 0.5$ Sum 29$	Bag 0.5 $ Burghul 3$ Oil 0.27 $ Tween 0.05$ Sum= 2.82	34.52
Material cost for local production per season ($) in 450 m²	14.5	87	2.82	104.32

The farmer's total cost of pesticides to control thrips, whiteflies spider mites and fungi is approximated at 550$ per season. An economic analysis shows that biological control is more expensive than the direct cost of the chemical control since the international price of the three BCAs used in for 450 m² greenhouse is 958 $. Although the international price of BCAs is higher, but that would be at safer environmental and reduced health risks, in addition to benefitting from better quality yield. However, if the production of the three BCAs is done in Lebanon, the cost may be reduced considerably, since rearing them in our

research labs costed us 104.32 $ for rearing materials for the BCAs to be released in the 450 m² greenhouse, excluding the cost of labor, rent, equipment, and other expenses.

In conclusion, although there were some significant difference between the population of thrips and whiteflies in the IPM greenhouse compared to the control, but, in both cases they were below economic threshold level with 14 insecticidal/acaricidal sprays in the control greenhouse and no insecticides nor acaricides sprays in the IPM greenhouse during the entire growing season. However, it should be noted that the pest populations of thrips and whiteflies increased significantly in May and June and that the release rates of the predator may be increased during this period based on periodic monitoring, once a week or even twice a week during June, when the average temperatures increases above 25, and become optimal for the development of whiteflies and thrips.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

In Lebanon, cucumber and pepper are considered to be two of the most important greenhouse-grown crops. Although greenhouse environmental conditions allow higher plant yield and better fruit quality, but they also favor an increase in the arthropod pests' populations. Farmers opt to spray toxic pesticides as it was the easiest way to control these pests. However, due to the excessive and repetitive pesticide applications, pests developed resistance to many pesticide families forcing farmers to apply pesticides more frequently, along with high environmental, health and economic costs. With the growing public awareness concerning pesticides, farmers in several developed countries are shifting to biologically based IPM strategies to suppress plant pest populations. Many success stories were published on the effectiveness of *A. swirskii* in controlling whiteflies and thrips which made it the most widely used biocontrol agent in protected cultivation worldwide within only 10 years (Knapp et al., 2018).

This research included two parts: Rearing of *C. lactis* (CL) and *A. swirskii* (SW) in the lab on different media for mass production of *A. swirskii*, and field trials for evaluating the efficacy of *A. swirskii* in controlling two insect pests on cucumber and pepper plants.

The four lab trials conducted for rearing *C. lactis* showed that the best medium was coarse autoclaved wheat bran with 20% yeast.

Depending on the market demand, if we need to harvest *C. lactis* at 1 WPI, 3,000 CL should be added to 1 g culture medium of 20% yeast, while if we want to harvest at 2 WPI, 750 CL should be added to this medium.

Five lab trials were performed to determine the suitable medium for rearing *A. swirskii*. Experiments 1, 2 and 3 proved that yeast is essential in the medium where *A. swirskii* is reared because they prey on *C. lactis* which needs yeast for its development. The most successful medium in the three trials was 20% yeast with 1:50 ratio (SW/CL) where *A. swirskii* were able to increase from 750 to 14,935 in 50g culture media within 4 weeks in trial 3. Trial 4 proved that the double spaced helped in the extension of the shelf-life of *A. swirskii* .However, in trial 5 no significant difference was recorded between the control where water was placed next to the culture to provide humidity and the treatment where calcium propionate was sprayed on the medium. Trial 6 proved that the addition of sucrose to the medium with calcium propionate will improve yield and extend the production cycle of SW.

The small-scale greenhouse test used to check the efficacy of the locally reared *A. swirskii* against their natural preys on cucumber plants showed that the two release rates 50 and 100/m² were effective in suppressing both whiteflies nymphs and eggs through the growing season with no significant difference between the two rates. *A. swirskii* was able to control and reduce significantly the number of whitefly eggs by 98 and 90% for treatments with 50 and 100/m² *A. swirskii*, ,respectively at 4 WPI, and the whitefly nymphs by 84 and 90% respectively with significant difference between the control and the two release rates of *A. swirskii* used, but without significant difference between the two release rates. The large-

scale greenhouse trial in Kfarmashoun was conducted in two commercial size greenhouses to determine the efficacy of *A. swirskii* in controlling thrips and whiteflies on cucumber and pepper plants. The introduction of the natural enemies, *A. swirskii* and *P. persimilis* (which will be reported in another thesis) were sufficient for controlling the pest populations during the growing season in the IPM greenhouse. With 7 introductions of *A. swirskii*, whiteflies and thrips were maintained below the ETL through the growing season on both cucumber and pepper. These results where similar to those recorded in the control greenhouse where 14 pesticide sprays, with a minimum of 2 active ingredients per spray, were used for controlling the whiteflies and thrips populations and keeping them below their respective ETLs; however, at high environmental, health and economic costs.

In conclusion, this study showed the possibility of mass rearing *A. swriskii* under laboratory conditions. and the efficacy of the reared local strain of *A. swirskii* in suppressing thrips and whiteflies to below ETLs, with 100% reduction in insecticide applications targeting these two pests on cucumber and pepper.

Recommendations:

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

1. Trying different artificial media for rearing *A. swirskii.*
2. Recommend to the Ministry of agriculture to conduct surveys in order to determine the beneficial organisms present in Lebanon
3. Mass rearing of different local natural enemies effective against different pests which will help farmers in reducing their pesticide sprays.

4. Repeating the experiment on another location, and during different growing seasons.
5. Testing the effectiveness of *A. swirskii* in controlling thrips and whiteflies on other major greenhouse crops such as tomato.
6. Recommend the application of pesticides that are not harmful to naturally occurring beneficial organisms.
7. Recommend to farmers to shift from the conventional way of controlling pests to biologically based- IPM strategies in order to reduce human and environmental risks and improve their working conditions

Appendix I: Tables of results of the small-scale experiment for evaluating the efficacy of local *A. swirskii* in controlling whiteflies

Table. I The average number of *A. swirskii* vs whitefly eggs on 6 leaves of 2 cucumber plants in 1m²

		Control	T1	T2
Week 3	SW	0	19	25
	WFE	21.66 ^{a}	0 ^{b}	0 ^{b}
Week 4	SW	0	28.33	41.33
	WFE	48.33 ^{a}	1 ^{b}	5 ^{b}
Week 5	SW	0	40.33	59
	WFE	32.33 ^{b}	2.66 ^{b}	1 ^{b}
Week 6	SW	0	7.66	3.66
	WFE	260 ^{a}	70.33 ^{b}	70 ^{b}

The average of whitefly eggs of the same week for different treatments with different alphabetical superscripts are significantly different with $p<0.05$.

Table. II The average number of *A. swirskii* vs whitefly nymphs on 6 leaves (2 cucumber plants) in cage of 1m².

		Control	T1	T2
Week 3	SW	0	19	25
	WFN	13.33 ^{b}	8.66 ^{b}	8^{b}
Week 4	SW	0	28.33	41.33
	WFN	95.33 ^{b}	31.66 ^{b}	43 ^{b}
Week 5	SW	0	40.33	59
	WFN	451.16 ^{a}	70.33 ^{b}	42 ^{b}
Week 6	SW	0	7.66	3.66

	WFN	148.33 ^{b}	41.66 ^{b}	50.33 ^{b}

The average of whitefly nymphs of the same week for different treatments with different alphabetical superscripts are significantly different with $p<0.05$.

APPENDIX II: Tables of results of large-scale greenhouse experiments for evaluation of the efficacy of *A. swirskii* for the management of two greenhouse cucumber pests: whiteflies and thrips in Kfarmashoun/Caza of Byblos

Table I. The average number of adult thrips per cucumber plant*

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Control	0.180	0.060	0.320	0.420	0.220	0.320	0.340	2.14 b	1.36 b	0.76 b	0.960	0.640	0 b
IPM	0.240	0.180	0.100	0.280	0.660	0.540	1.020	4.64 a	3.15 a	2.42 a	1.020	0.480	0.46 a
SEM	0.046	0.036	0.059	0.089	0.115	0.099	0.109	0.638	0.248	0.260	0.169	0.108	0.068

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table II. The average number of thrips larvae per cucumber plant*

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Control	0.460	0 b	0 b	0.22 b	0.34 b	1.08 b	7.12 b	1.32 b	3.54 b	3.04 b	1.68 b	4.74 a	0 b
IPM	0.700	0.36 a	0.32 a	0.92 a	1.92 a	5.06 a	14.46a	19.1 a	14.7 a	11.3 a	3.14 a	1.3 b	0.54 a
SEM	0.159	0.082	0.051	0.163	0.292	0.491	1.104	1.797	1.186	1.161	0.339	0.774	0.080

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table III. The average number of whiteflies adult per cucumber plant*

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Control	0.020	0.000	0.000	0.160	0.500	0.260	0.600	1.020	1.260	1.800	0.66 b	4.200	8.02 a

IPM	0.140	0.160	0.260	0.280	0.560	0.160	0.460	1.960	1.225	2.880	4.86 a	4.980	3.06 b
SEM	0.046	0.051	0.081	0.095	0.164	0.074	0.225	0.533	0.228	0.418	0.972	1.012	0.965

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table IV. The average number of whiteflies nymphs per cucumber plants

Average in a column with different alphabetical superscripts are significantly different with

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Control	0.000	0.000	0.020	0.000	0.080	0.000	0.000	0.24 b	0.560	0.3 b	0.02 b	8.160	14.5 a
IPM	0.000	0.000	0.000	0.000	0.000	0.000	0.320	2.38 a	0.950	3.32 a	4.22 a	3.580	0.3 b
SEM	0.000	0.000	0.010	0.000	0.040	0.000	0.112	0.501	0.286	0.549	0.852	1.378	2.578

p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table V. The average number of *A. swirskii* per cucumber plant*

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Control	0.000	0.000	0 b	0.000	0.000	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
IPM	0.000	0.020	0.08 a	0.020	0.040	0.96 a	2.18 a	9.86 a	18.175 a	16.204 a	34.76 a	53.08 a	76.52 a
SEM	0.000	0.010	0.020	0.010	0.020	0.117	0.218	1.418	2.120	1.681	2.565	3.971	5.476

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Pepper:

Table VI. The average number of adult thrips per pepper plant*

	1	2	3	4	5	6	7	8	9	10	11	12
Control	1.567	0.400	0.333	2.466a	2.400	1.467	1.533 b	4.866 a	3.533 a	1.167	3.200	3.567

IPM	1.067	0.385	0.433	0.7 b	1.621	1.833	3.433 a	2.666 b	1.866b	1.733	2.667	1.867
SEM	0.142	0.094	0.089	0.246	0.262	0.221	0.377	0.573	0.291	0.236	0.619	0.504

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table VII. The average number of larvae thrips per pepper plant*

	1	2	3	4	5	6	7	8	9	10	11	12
Control	0.267	0.067	0.033	0.633	0.633	1.533	1.833	2.9 a	1.4 a	0.600	1.667	1.2 a
IPM	0.033	0.033	0.100	0.600	0.667	0.800	1.200	1.066 b	0.533 b	0.833	0.267	0.066 b
SEM	0.066	0.037	0.040	0.126	0.130	0.206	0.239	0.411	0.204	0.133	0.551	0.166

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table VIII. The average number of whiteflies adults per pepper plant*

	1	2	3	4	5	6	7	8	9	10	11	12
Control	0.000	0.000	0.133	0.300	0.033	0.067	0.0000 b	0.000	0.367	0.300	0.700	0.533
IPM	0.000	0.633	1.000	0.233	0.000	0.133	0.9 a	1.533	0.767	0.867	1.533	1.900
SEM	0.000	0.182	0.232	0.109	0.017	0.052	0.206	0.470	0.370	0.348	0.482	0.553

Average in a column with different alphabetical superscripts are significantly different with p<0.05. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table IX. The average number of whiteflies nymph per pepper plant*

1	2	3	4	5	6	7	8	9	10	11	12

Control	0.067	0.000	0.000	0.167	0.100	0.200	0.000 b	0.067	0.000	0.600	0.633	0.433
IPM	0.133	0.000	0.633	0.000	0.300	0.333	0.7 a	0.467	0.167	1.000	0.467	0.233
SEM	0.057	0.000	0.226	0.068	0.142	0.142	0.150	0.106	0.060	0.260	0.287	0.172

Average in a column with different alphabetical superscripts are significantly different with $p<0.05$. *Average of 50 plants per group, **SEM: Standard Error of the Mean

Table X. The average number of *A. swirskii* per pepper plant*

	1	2	3	4	5	6	7	8	9	10	11	12
Control	0 b	0 b	0 b	0.000	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
IPM	0.4 a	0.366 a	0.833a	0.033	0.233 a	0.7 a	1.033 a	1.233 a	3.033 a	2.4 a	3.6 a	4.4 a
SEM	0.066	0.090	0.129	0.017	0.059	0.123	0.146	0.160	0.359	0.343	0.377	0.586

Average in a column with different alphabetical superscripts are significantly different with $p<0.05$. *Average of 50 plants per group, **SEM: Standard Error of the Mean

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