Control of the Mango Shield Scale, *Milviscutulus Mangiferae* (Green) by Two Bio Rational Insecticides

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ABSTRACT

Verticillium lecanii **(Zimm) conidia were formulated in our laboratory (a myco-insecticide), WP (2.5%) and tested for its reduction on the mango shield scale,** ***M. mangiferae*** **(Green) different stages on the host plant trees comparing with a commercial insecticide (Pyriproxyphen (Admiral), 10% EC) for their reduction of each** ***M. mangiferae*** **stage as well as its total population. All the treated insect stages populations were differently affected in a function of the treated stage and the time after treatment (exposure time). The formulated bio-insecticide (myco-insecticide) was significantly more toxic than pyriproxyfen (admiral) in its effect on adult population especially after two weeks of treatment during the two season's treatment. It showed an effect similar to the pyriproxyfen (admiral) against the other stages. Both the 1st and the 2nd nymph instars were less affected than the other treated stages (adult and crawler) with the two tested bio-insecticides. The low toxicity of the formulated** ***V. lecanii*** **to non-target biota encouraged us to stress on its entrance to the** ***entomopathogenic*** **fungi clique against the examined insect,** ***M. mangiferae*****.**

Key words: ***Verticillium lecanii*****,** ***Milviscutulus mangiferae*****, Pyriproxyphen (admiral).**

INTRODUCTION

Mango, *Mangifera indica* L. (Anacardiaceae) is a popular fruit in Egypt occupying economic importance worldwide for its flavor and taste (Karar *et al*., 2015). The mango shield scale is a polyphagous, feeding on plants assigned to more than 86 genera (more than 43 botanical families). It is a serious pest of mango (*M. indica*) (Malumphy, 2018).

Infestation on the mango trees with the mango shield scale, *M. mangiferae* (Green) (Hemiptera: Coccidae) destruct the fruit crop (Abd-Rabou & Evans, 2018; Attia *et al.*, 2018 and El-Baradey *et al.*, 2020) through sucking the plant sap with the mouth parts, this insect injures the shoots, twigs, leaves, branches and fruits causing small sized leaves with chlorosis, deformations, defoliation, drying up of young twigs, dieback, poor blossoming reaching the death of twig and branches by its saliva toxicity (Grimshaw & Donaldson, 2007; Soliman *et al*., 2007; Hassan *et al.*, 2012 and Bakry *et al*., 2013). This insect also covers plant leaves with its excretion of a large honeydew amount attracting ants on leaves and encouraging the growth of sooty mold fungus that exhibits dirty black appearance reducing photosynthesis and respiration as well as lowering the fruit quality causing considerable economic loss (Atalla *et al*., 2007 and Nabil, 2013). Having information about density and changes in population fluctuations of *M. mangiferae* during the year will help mango producers in this pest management. Mohamed (2020) indicated that *M. mangiferae* is active all the year with different individual numbers with three peaks of activity on mango trees for total population in January, July and November. Parthenogenesis reproduction of *M. mangiferae*, walking all stages over the host to a suitable place to settle and feed (Kasuya, 2000), and morphologically adaptation for passive dispersal by wind help its wide spread and danger. Pyriproxyfen (IGR) controlled CRS with low toxicity on its primary parasitoid, *Aphytis melinus* DeBach (Rill *et al*., 2008 and Rezk *et al*., 2021).

Due to insect resistance and environmental risks of the conventional chemical pesticides, new alternatives as biological control for insect pest management was favored (Upadhyay *aet al*., 2014). *V. lecanii* (Zimm.) Viegas acts as a promising control agent for various insect pests. The high virulence and epizootic efficiency of some *V. lecanii* strains towards certain insects was used for developing commercial myco-insecticides to control of aphids, whiteflies and other sucking insects (Zare *et al*., 2000; Gams & Zare, 2001 and Aiuchi *et al*., 2008).

So, in this study, an insect growth regulator (pyriproxyphen (admiral), 10% EC) and our formulated *V. lecanii* (a bio-insecticide), WP 2.5% were test regarding their reduction of each *M. mangiferae* stage as well as its total population on mango host plant trees on December 2021 and December 2022 as the treated insect has three peaks of activity on mango trees.

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MATERIALS AND METHODS

Treated Insect

The mango shield scale, *M. mangiferae* (Green) as the most spreader in the treatment region.

Identification of the treated insect

The treated insect, *M. mangiferae* (Green) was identified on the host plant trees through photographing with a stereomicroscope at 40X magnification comparing with Hamon and Williams (1984).

Preparing the *V. lecanii* formulation

The entomo-pathogenic fungus, *Verticillium lecanii* (Zimm) Viegas that was originally isolated from Alaska, USA through Egypt microbial culture collection (EMCC) under the number: 919™ was used in this study. This fungus was obtained as an isolate only and we produced its conidia mass production under our conditions. This fungus was formulated in a wettable powder (WP) formulation (2.5% WP). The silica gel powder (the carrier material) was sieved through 355 mesh size to maintain uniformity of the carrier material particle size with the conidial powder. This carrier material was sterilized in an autoclave at 120°C and 15 Psi for 30 min and mixed with conidial powder after two days. A weight of 2.5 gm of fresh *V. lecanii* conidial powder at a concentration of 2×10^9 conidia/ml conidia were mixed with the sterilized silica gel powder at 2.5:10 weight ratios. Both talc powder and starch were added as fillers up to a final total weight of 100.0 gm. Casein UV-protectant was added at the rate of 2 % by weight and the wetting agent Tween 80® was also added to the mixture of conidia at 0.5 % by weight. Carboxy methyl cellulose (CMC) was added at a rate of 2 % by weight as a sticker. All materials were mechanically mixed.

Tested bio-insecticides

Both the fungus originated myco-insecticide and the insect growth regulator, pyriproxyphen (admiral, 10% EC) were tested against the treated insect, the mango shield scale, *M. mangiferae* (Green) different stages. Their names, application and other features are tabulated below.

Experimental procedure

All insect stages were treated with foliar application on the fruity mango trees in Rashid City, Behira Governorate, Egypt. The host mango trees were not chemically treated two years before this study. The treatment was randomized complete block (RCBD) design. Four trees were taken for each replicate and four replicates were used for each treatment. The insect stages were sprayed once with the tested materials at the application rates with the 20-liter sprinkler machine. Fifty (50) leaves of each replicate were randomly taken directly before spraying (pre-spraying) for determining the infestation limit and 2, 4, 6 and 8 weeks after treatment in treatment and control. These leaves were transferred in paper bags to the laboratory and each stage of the treated insect was individually counted at each time interval. The total number was also calculated. Counting and discriminating the alive number of each stage were carried out using the stereomicroscope. Counting was repeated four times in each replicate and its mean number was calculated. The four mean numbers of the four replicates were averaged for each treatment. Control was currently conducted. The reduction percentage in each studied stage and the total population was calculated according to Henderson and Tilton (1955) formula.

$$\text{Reduction \%} = 100\ [1- (T_2 / T_1 * C_1 / C_2)]$$

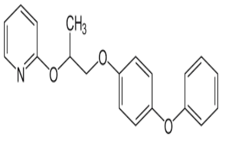
T_2 Population in Treatment after spray

T_1 Population in Treatment before spray

C_1 Population in control before spray

C_2 Population in control after spray

The tested insecticides against *M. mangiferae* (Green) stages

Common name (Trade name)	Chemical Class	Manufacturer	Application rate	Chemical structure
Pyriproxyphen (Admiral) 10% EC	Phenoxypheny lpyridyloxy) propyl ether	Sumitomo Chemical Australia Pty Ltd A.B.N. 21 081 096 255	50 ml / 100 L	
Verticillium lecanii WP 2.5%		Originally isolated from Alaska, USA EMCC Number: 919™	500 gm / 100 L	

Statistical Analysis

The tested materials (pyriproxyfen (admiral) and *V. lecanii*) treatments resulted data were analyzed (Costat, 2005). The least significant difference at 0.05% (LSD $_{0.05}$) was used to compare the treatment means.

RESULTS AND DISCUSSION

Identification of the treated insect

All *M. mangiferae* (Green) stages (eggs, crawlers, adults, the first instar and the second instar) as well as some details of the insect body (anal setae, margin setae, anal plates and margin) were discriminated and photographed (Figure 1). This identification of the insect was done by Dr. Khadega Morsy, Professor, Scale insect and Mealy bugs Department, Plant Protection Research Institute, Sabahia Plant Protection Station, Agriculture Research Center, Alexandria, Egypt.

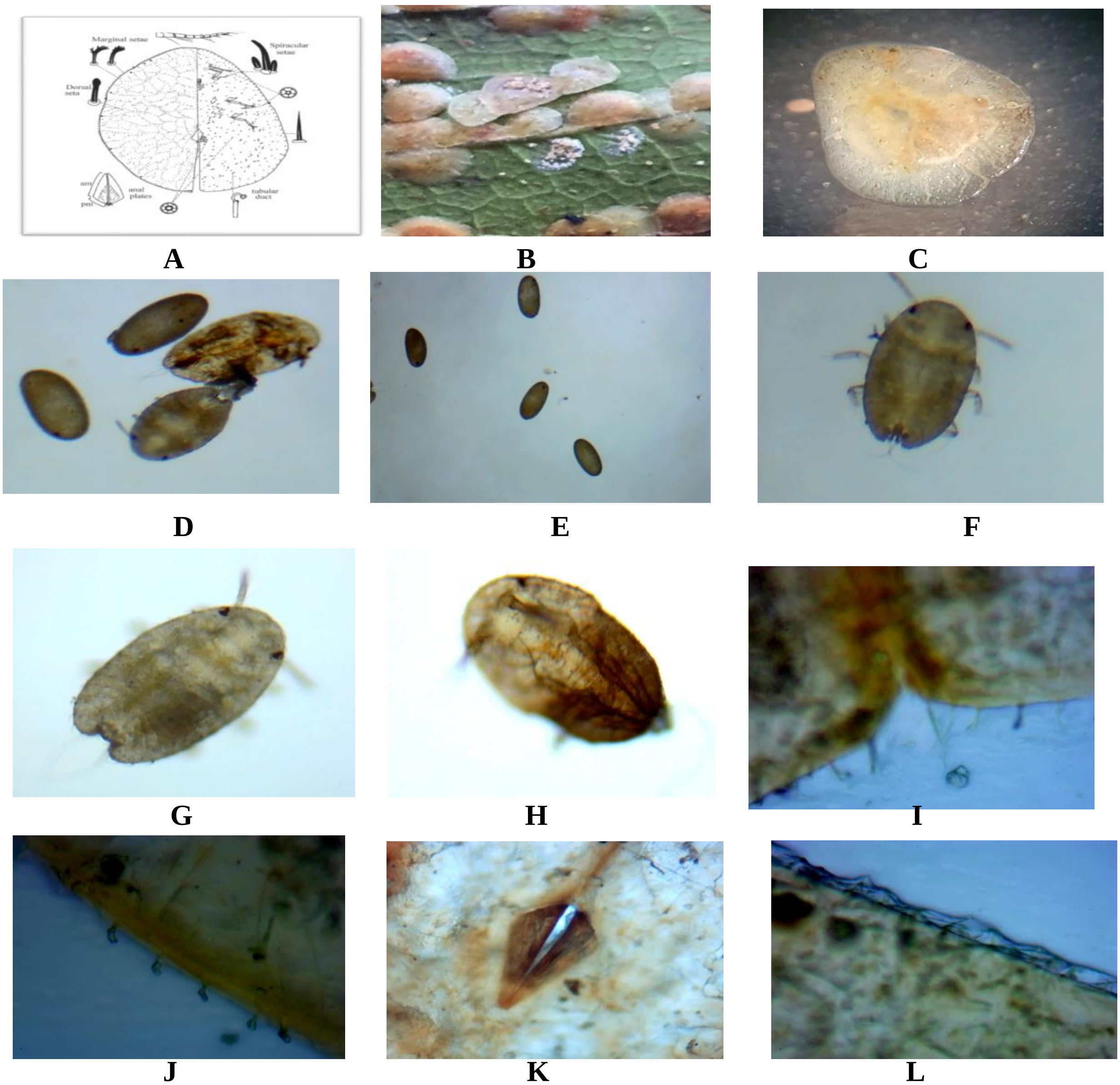


Fig. 1. Photo guides of the mango shield scale, *M. mangiferae* (Green)

A, *M. mangiferae* adult female (Hamon and Williams, 1984); B, *M. mangiferae* on mango leaves; C, *M. mangiferae*; D, different stages of *M. mangiferae*; E, Eggs; F, Crawler; G, First instar; H, Second instar; I, Anal Setae; J, Margin Setae; K, Anal plates; L, Margin.

Insecticidal activity of the tested materials

The obtained results showed the effects of the two tested bio-insecticides (with different modes of action). On the different treated insect stages populations, different effects in a function of stage, bio-insecticide and the exposure time were exhibited. Pyriproxyfen (admiral) appeared less effective against the *M. mangiferae* adult stage population with 46.71– 69.97% and 42.50 – 70.70% reduction ranges in 2021 and 2022 treatments, respectively in non-systematic arrangement with the exposure time. The formulated bio-insecticide was more effective than the used admiral with reduction ranges of 59.12 – 74.06% and 57.31– 71.54% in 2021 and 2022 treatments, respectively. Decreasing the activity was differed between the tested bio-insecticides and the formulated bio-insecticide appeared more potent after 8 weeks of treatment without any worry about the environment. Worth mentioning, the formulated bio-insecticide significantly overcame the pyriproxyfen (admiral) in its effect on adult population especially after two weeks of treatment in the two season's treatments (Table 1).

Against the treated *M. mangiferae* crawler population, the tested two bio-insecticides showed the same trend of activity by pyriproxyfen (admiral) and the formulated *V. lecanii* bio-insecticide. They achieved 75.18 – 89.57% and 75.47– 85.14% reduction ranges, respectively in 2021 treatment in non-systematic arrangement within the experimental time in comparison to 77.98 – 87.53% and 75.00– 86.65% in 2022 treatment in the same array. The *V. lecanii* formulated bio-insecticide affected the crawler population similarly as the used pyriproxyfen (admiral) at two weeks' exposure after treatment in 2021 and at 2,6 and 8 weeks after treatment in 2022, respectively. The effect of both tested bio-insecticides was decreased at eight weeks after treatment with no significance between them specially in 2022 treatment (Table 2).

Table 1. Effect of *V. lecanii* on *M. mangiferae* adult females comparing with pyriproxyfen (admiral); shown as average reduction%± SD

Tested toxicant	Reduction % in *M. mangiferae* adult number after treatment (weeks)							
	Season ٢٠٢١ Treatment				Season 2022 Treatment			
	2	4	6	8	2	4	6	8
Pyriproxyfen (Admiral)	46.79± 8.58 b	69.97± 4.10 a	68.77± 7.73 a	57.41± 5.37 b	42.50± 2.19 b	66.45± 2.04 b	70.70± 3.65 a	60.16± 2.12 a
Verticillium lecanii	59.12± 0.66 a	73.82± 5.34 a	74.06± 4.56 a	65.74± 5.56 a	57.31± 4.89 a	71.54± 4.16 a	68.68± 2.35 a	62.11± 3.65 a
LSD $_{0.05}$	5.89	3.98	6.32	5.68	4.29	3.31	3.64	4.83

Means in the same column (s) with the same letter are not significant at 0.05 level of probability.

Table 2. Effect of *V. lecanii* on *M. mangiferae* crawler comparing with pyriproxyfen (admiral); shown as average reduction%± SD

Tested toxicant	Reduction % in *M. mangiferae* crawler number after treatment (weeks)							
	Season ٢٠٢١ Treatment				Season 2022 Treatment			
	2	4	6	8	2	4	6	8
Pyriproxyfen (Admiral)	75.18± 2.44 a	88.61± 1.35 a	89.57± 1.28 a	85.46± 3.62 a	78.24± 3.25 a	87.53± 2.35 a	87.00± 0.67a	77.98± 1.96 ab
Verticillium lecanii	75.47± 2.88 a	83.66± 2.65 b	85.14± 2.15 b	79.50± 2.13 b	75.60± 3.65 a	84.41± 2.25 b	86.65± 2.35 a	75.00± 2.67 b
LSD $_{0.05}$	2.80	2.41	1.54	3.15	4.27	2.45	2.06	3.05

Means in the same column with the same letter are not significant at 0.05 level of probability.

Table 3. Effect of *V. lecanii* on *M. mangiferae* 1^{st} instar nymph comparing with pyriproxyfen (admiral); shown as average reduction%± SD

Tested toxicant	Reduction % in *M. mangiferae* 1^{st} instar number after treatment (weeks)							
	Season ٢٠٢١ Treatment				Season 2022 Treatment			
	2	4	6	8	2	4	6	8
Pyriproxyfen (Admiral)	55.83± 4.07 b	77.82± 4.53 a	82.16± 2.66 a	69.95± 3.43 a	56.29± 3.04 a	81.37± 2.67 a	83.47± 3.76 a	78.83± 2.44 a
Verticillium lecanii	65.93± 5.53 a	67.97± 5.39 b	71.60± 3.81 b	65.35± 5.96 a	59.70± 3.60 a	69.61± 4.13 b	71.08± 3.23 b	71.19± 3.07 b
LSD $_{0.05}$	5.54	4.69	3.96	5.10	4.13	3.37	4.46	3.67

Means in the same column with the same letter are not significant at 0.05 level of probability.

Table 4. Effect of *V. lecanii* on *M. mangiferae* 2^{nd} instar nymph comparing with pyriproxyfen (admiral); shown as average reduction%± SD

Tested toxicant	Reduction % in *M. mangiferae* 2^{nd} instar number after treatment (weeks)							
	Season ٢٠٢١ Treatment				Season 2022 Treatment			
	2	4	6	8	2	4	6	8
Pyriproxyfen (Admiral)	52.13± 2.35 a	74.11± 1.63 a	77.99± 3.67 a	70.42± 3.92 a	53.50± 1.35 a	76.94± 2.00 a	79.53± 1.80 a	80.13± 2.27 a
Verticillium lecanii	56.61± 2.78 a	62.56± 4.16 b	65.24± 2.67 b	66.76± 2.57 b	50.00± 7.64 a	64.82± 3.39 b	66.96± 2.86 b	68.52± 3.91 b
LSD $_{0.05}$	4.83	3.65	3.28	2.71	5.75	4.08	2.91	3.37

Means in the same column with the same letter are not significant at 0.05 level of probability.

The two tested bio-insecticides affected the first and the second instars of the *M. mangiferae* in a lesser degree comparing with the other treated stages. Against the 1^{st} instar, pyriproxyfen (admiral) caused 55.83 – 82.16% and 56.29 – 83.47% reduction in the treated population in the same array. The formulated *V. lecanii* killed the 1^{st} instar of the *M. mangiferae* insect with 65.35 - 71.60% and 59.70 – 71.19% reduction ranges in the same array. The two tested bio-insecticides exhibited their lethality in non-systematic arrangement with the time of exposure after treatments and between the tested bio-insecticide and the used reference IGR pyriproxyfen (admiral) during the evaluation period (Table 3).

The same trend of the tested bio-insecticide effect was noticed against the 2^{nd} nymph instar of the treated insect population as they exhibited their lethal effects on it in non-systematic arrangement with the time after treatment. Pyriproxyfen (admiral) caused 52.13 – 77.99% and 53.50 – 80.13% reduction in 2021 and 2022 treatments, respectively. The formulated used bio-insecticide (*V. lecanii*) affected the second instar significantly as similar as the pyriproxyfen (admiral) compound as it caused 56.61– 66.76% and 50.00 – 68.52% reduction ranges in 2021 and 2022 treatments in the same array. Worth mentioning, the tested bio-insecticide *(V. lecanii*) appeared less effective against the second nymph instar of *M. mangiferae* insect than the used reference compound except after two weeks exposure, there was no significance between them in the two seasons treatments (Table 4).

As a general, the total *M. mangiferae* stages census was diminished because of treatments with the used materials (the formulated used bio-insecticide (*V. lecanii*) and the used reference insecticide) in the two seasons (2021 and 2022) treatments. The results are recorded in Table (**5**), from which it could be deduced that pyriproxyfen (admiral) and the formulated used bio-insecticide (*V. lecanii*) achieved insecticidal effects against the treated insect total stages number ranged from 59.87–82.28% and 59.95 – 80.96% in comparison to 65.37– 75.99% and 62.17 – 75.21% reduction, respectively in 2021 and 2022 treatments. Although the tested fungus originated insecticide significantly overcame the used reference insecticide at two weeks after treatment in 2021 treatment with no significance between them in 2022 treatment at the same exposure time, it appeared less effective than it at 4,6 and 8 weeks after treatments in both treatments.

Table 5. Effect of *V. lecanii* on *M. mangiferae* total number comparing with pyriproxyfen (admiral); shown as average reduction%± SD

Tested toxicant	Reduction % in *M. mangiferae* total stages number after treatment (weeks)							
	Season ٢٠٢١ Treatment				Season 2022 Treatment			
	2	4	6	8	2	4	6	8
Pyriproxyfen (Admiral)	59.87± 1.46 b	79.54± 1.77 a	82.28± 0.48 a	74.69± 1.20 a	59.95± 1.40 a	79.18± 1.29 a	80.96± 0.83 a	74.74± 1.72 a
Verticillium lecanii	65.37± 2.89 a	74.04± 1.26 b	75.99± 1.72 b	71.02± 1.29 b	62.17± 2.03 a	74.04± 2.55 b	75.21± 0.47 b	69.71± 1.06 b
LSD	2.73	1.59	1.54	1.23	2.51	2.13	0.96	1.94

Means in the same column with the same letter are not significant at 0.05 level of probability.

From the obtained results, it could be concluded that the formulated bio-insecticide of *V. lecanii* exceeded the pyriproxyfen (admiral) in its effect against some stages at some exposure times. All of these differences in the effect are due to different modes of action of the applied bio-insecticides, which may control the time required to kill the treated insect stage but all of the tested insecticides persuaded us for their satisfactory effect. Pyriproxyphen (admiral) appeared less effective on adult than on other nymphal and crawler stages because of its IGR mode of action, which sterilizes the adults and causes nymphal reduction. It disrupts the molting through chitin synthesis inhibition. Since this material affects molting, treatment should be made during peak crawler (1st instar) emergence (Cruz *et al*., 2013).

Regarding our formulation of *V. lecanii*, it exceeded the IGR used compound in some cases with different mode of action through its hydrophilic conidia (Inglis *et al*., 2001) adsorption to the surface through the recognition of specific glycoprotein receptors in the insect, adhesion or consolidation the interface between the pre-germinated spores and the epicuticle as well as germination and development until oppressor formation initiating the penetration phase (Tellez-Jurado *et al*., 2009). The high virulence and epizootic efficiency of some *V. lecanii* strains towards certain insects has been used to develop commercial myco-insecticides as mycotal, vertalec and biovert for the biological control of aphids and whiteflies. The mechanism of action of the fungus conidial spores may be through attaching to the insect cuticle and germination, followed by penetration the insect's integument by the germinated spore's hyphae destroying the insect internal contents (Zare *et al*., 2000 and Gams & Zare, 2001).

On the other sight, these mycelia produce toxin with insecticidal properties weaken the insect immune system and aid in eventually killing it. Infected insects appear as white to yellowish cottony particles. It is revealed that the fungus infects the insect on contact and does not need to be consumed by the host to cause infection (Badii and Abreu, 2006).

Environmentally, the *V. lecanii* is not phytotoxic, not accumulated in plants and fruits, ensures eco-friendly and safe products, can be applied at any stage of plant growth, no safety interval, recommended for prophylactic use and within integrated plant protection systems and safe for people, warm-blooded animals, hydrobionts, bees and entomo-phages. It has been recently commercialized as a potential bio-control agent for pest management enjoying compatibility with commonly used agrochemicals and other bio-control agents (Upadhyay *et al*., 2014). So, based on the obtained result and its environmental behavior, our formulation of *V. lecanii* was found less toxic on mammals, predators and other non-target biota encouraging us to stress on their entrance the *entomopathogenic* clique against the examined insect, *M. mangiferae*.

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الملخص العربي

مكافحة حشرة المانجو القشرية *M. mangiferae* (Green) حقلياً بواسطة مبيدين حيويين

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تم تجهيز كونيديا فطر الـ *Verticillium lecanii* (Zimm) فى صورة مسحوق قابل للبلل (٢,٥%) فى معملنا كمبيد حيوى من الفطر واختباره كمبيد حيوى لإبادة جميع أطوار حشرة المانجو القشرية *Milviscutulus mangiferae* (Green) على أشجار المانجو مقارنة بالمبيد الحشرى المعروف الأدميرال (البيريبروكسيفين،EC ١٠%) فى قدرتهما على إختزال تعداد كل طور من أطوار الحشرة بالإضافة إلى التعداد الكلى لهذه الآفة.

جاء التأثير على الأطوار المختلفة لهذه الحشرة كدالة فى كل من الطور المعامل، المبيد الحيوى المستخدم بالإضافة إلى زمن التعرض للمبيد المختبر.

ثبت أن المبيد المجهز من الفطر قد تعدى المبيد المستخدم القياسى فى تأثيره على طور الحشرة الكاملة خاصة بعد أسبوعين من المعاملة فى موسمى ٢٠٢١ و ٢٠٢٢، كما ظهر مشابهاً فى درجة تأثيره مع مركب الأدميرال المستخدم على بقية الأطوار. وجد أيضا أن الطور الأول والثانى للحوريات Nymph ظهرا أقل تأثراً من بقية الأطوار بهذين المركبين. ظهرت سمية هذين المركبين متزايدة مع زيادة زمن التعرض و لكن ليست بصورة ثابتة التغير مع زيادة زمن التعرض.

تعد النتائج المتحصل عليها بالإضافة إلى السمية المنخفضة على الكائنات الحية الغير مستهدفة مشجعاً هاماً لإدخال هذه التجهيزة لكونيديا فطر الـ فيرتيسيليوم ليكانيـاى *Verticillium lecanii* (Zimm) ضمن مجموعة الفطريات القاتلة للحشرات ضد الحشرة محل الدراسة، *M. mangiferae*.