

THE EFFECT OF DIFFERENT BIOPESTICIDES ON THE CABBAGE WHITE BUTTERFLY, *PIERIS RAPAE* (L.) IN CAULIFLOWER FIELDS

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Received on: 14/1/2009

Accepted: 1/3/2009

ABSTRACT

The cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae) is a dangerous pest of many foliar vegetable crops, particularly cauliflower. Entomopathogenic nematodes (EPNs), entomopathogenic fungi (EPF) and synthetic insecticides such as Proclaim and Spinosad are common biopesticides used nowadays in sustainable agriculture. The main effect of these bioinsecticides is significantly different as to the mortality of larvae and pupae of *P. rapae*. With respect to nematode isolates and/or strains belonging to *Heterorhabditis* spp. (B 20 & H 44) and *Steinernema* spp. (At s & At4), no different effects occurred between these strains in the larval stage. While highly significant effects were obtained with the pupal stage. Entomopathogenic fungi belonging to *Beauveria bassiana* (F1, F2, and F3) exhibited moderate effect on the larvae of *P. rapae*, while it was high on the pupae. The larvae of *P. rapae* were highly more sensitive to spinosad than proclaim, while the pupal stage was less sensitive to both synthetic pesticides. Under field conditions the results indicated that spinosad provided a therapeutic and residual level of control against *P. rapae*, These were equivalent or superior to other tested biopesticides. Spinosad and *Steinernema* spp. (At 4) were similar, while both were superior to other biopesticides. Meanwhile, proclaim and *Heterorhabditis* spp. (B 20) came in the second grade of reducing the population of *P. rapae* under field conditions. Moderate insect population reduction was obtained by *B. bassiana* (F 2), while the least insect population reduction occurred with *B. bassiana* (F 1).

Key words: Entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, entomopathogenic fungi, *Beauveria bassiana*, *Pieris rapae*, Proclaim, Spinosad,

INTRODUCTION

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. They are an important group of pesticides that can reduce the side effect of insecticides. Biopesticides, in general: 1) Have a narrow target range and a very specific mode of action; 2) Are slow acting; 3) Have relatively critical application times; 4) Suppress, rather than eliminate, a pest population; 5) Have limited field persistence and a short shelf life; 6) Are safer to humans and the environment than conventional pesticides; 7) Present no residue problems. The two types of biopesticides are biochemical and microbial. Biochemical pesticides may have a similar structure to, and function like, naturally occurring chemicals, and have nontoxic modes of action. Insect pheromones, for example, are naturally-occurring chemicals that insects use to locate mates. Man-made pheromones are used to disrupt insect mating by creating confusion during the search for mates, or can be used to attract male insects to traps. Pheromones are often used to detect or monitor insect populations, or in some cases, to control them. Microbial insecticides are another kind of biopesticide. They come from naturally-occurring or genetically altered bacteria, fungi, algae, viruses or protozoans. They suppress pests by: 1) Producing a toxin specific to the pest; 2) Causing a disease; 3) Preventing establishment of other microorganisms through competition; or 4) Other modes of action.

Entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) are parasites of insects that kill their hosts with the aid of bacteria carried in the nematode's

alimentary canal; steinernematids carry *Xenorhabdus* spp. whereas heterorhabditids carry *Photorhabdus* spp. (Adams and Nguyen, (2002) and Poinar, (1990). These nematodes can be used as biological control agents to suppress a variety of economically important insect pests (Grewal et al., 2005; Shapiro-Ilan, 2004). No matter how well suited an entomopathogenic nematode is to a targeted pest, the application will fail if the agent is not delivered in a manner that enables access to and infection of the host. nevertheless, the technical aspects of biopesticide application in the field are often neglected. Effective and efficient delivery of entomopathogenic nematodes can only be achieved with careful consideration of available application technology coupled with an understanding of the attributes and limitations of the biocontrol agent. Entomopathogenic nematodes have been applied using practically every device available in the agricultural and urban environment, from knapsack sprayers.

A diverse spectrum of entomopathogenic fungi is reported from insects and mites (Goettel et al., 2005). Commercial development of entomopathogenic fungi has been confined to species in the Hypocreales, most notably *Lecanicillium* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus*. In general, these fungi are inundatively applied to bring about a rapid reduction in the pest population. Because fungi gain access to the host through the cuticle, they are the principal pathogens of sucking insects (Hemiptera, e.g., aphids and whiteflies) and control a variety of insects in other orders such as Coleoptera, Lepidoptera, and Orthoptera (Goettel et al., 2005).

Spinosad is a fermentation product produced by one or more chemical mutants of the naturally occurring actinomycetes soil bacterium *Saccharopolyspora spinosa* (Boek et al., 1994). Vegetative inoculum is grown by a submerged aerobic fermentation process. Spinosad has been applied to over 200 different crops. It has been used to control caterpillars in cotton, loppers or the cabbage butterfly, leaf miners in various crops, leaf rollers on apples, thrips on citrus, etc. (Thompson et al., 2000). Technical spinosad is especially insecticidal to small caterpillars by ingestion and contact, but especially by ingestion. It is not a plant systemic, but will penetrate leaves. Thus, it is active against leafminers and has activity against flies and thrips. On crops, higher application rates are needed to control thrips and leafminers more than for caterpillars.

Proclaim is a highly potent, insecticide unique foliar insecticide that controls lepidopteran pests (caterpillars and worms) in Cole crops, turnip greens, and leafy and fruiting vegetables. As the second most damaging insects behind sucking pests for vegetables crops, lepidopterous pests can significantly reduce crop's quality and yield. Proclaim effectively controls the larval stages of these pests at low rates, thereby increasing the crop's value. Proclaim contains the active ingredient emamectin benzoate, a semi-synthetic second generation avermectin insecticides. Proclaim can be applied by ground air or ground only, giving growers the flexibility needed for effective Integrated Pest Management (IPM) programs (Jansson, et al., 1997).

The present work was carried out to study the effect of different biopesticides (entomopathogenic nematodes, entomopathogenic fungi, and synthetic biopesticides "proclaim and spinosad") on the cabbage white butterfly, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) on cauliflower plants under field conditions.

MATERIALS AND METHODS

1- Target insect:

The cabbage white butterfly, *P. rapae* is a lepidopterous insect causing damage to many foliar vegetable crops especially cauliflower, which is a garden vegetable belonging to the mustard family, Brassicaceae or Cruciferae. Its scientific name is *Brassica oleracea* var. *botrytis*. Different larval stages and pupae were collected from field infestation for laboratory bioassay. The main goal of this bioassay was to study the efficacy of the biopesticides on the target insect, and as well as the contact effect of synthetic biopesticides (proclaim and spinosad 50g/feddan of each "0.05%") on the larval and pupal stages.

2- Biocontrol agents :

Four isolates and/or strains of Egyptian entomopathogenic nematodes were tested, of which two belonging to *Steinernema* genus (At s & At 4

strains) and the other two belonging to *Heterorhabditie* genus (B20 & H44 strains). Three isolates of local entomopathogenic fungi (F1, F2, & F3) of *B. bassiana* were used. Also, two commercial formulations of synthetic biopesticides (Spinosad & Proclaim) were also tested.

3- Entomopathogenic fungi (EPF) culture :

The three isolates of EPFs were grown with solid media on Sabouraud dextrose yeast agar containing, 1% peptone, 0.2 % yeast extract, 4% dextrose and 1.5% agar in distilled water at $23 \pm 3^\circ\text{C}$ for at least two weeks. Conidiospores were harvested and suspended in sterile distilled water containing 0.05% Tween 80 from 14 days old cultures under sterile conditions (Shamseldean et al., 2003). The concentration of fungal spores 5×10^7 spores/ml mixed with 10 % of soya bean milk solution was used for field experiment.

4- Entomopathogenic nematodes (EPNs) culture :

Two nematode isolates and/or strains of *Steinernema* spp. (Ats & At4), and two *Heterorhabditis* spp. (B20 & H44) were cultured on the last instar larvae of *Galleria mellonella* (L) according to the method by Dutky et al. (1964) and IJs were harvested from nematode traps as described by White (1927) at $25 \pm 2^\circ\text{C}$. A stock suspension of the IJs (for laboratory and field experiments) in sterilized distilled water was stored at 15°C until required. All nematodes were used within 2 weeks of harvest and a new infection cycle and a stock of IJs were prepared every 2 weeks.

5- Laboratory experiment :

In the laboratory experiment, the last instar larvae and pupae of *P. rapae* were tested under laboratory conditions using glass Petri dishes (2 cm high, 15 cm in diameter) covered with lid. Each dish contained part of cauliflower leaf dipped in a suspension of the tested bioagent. Three replicates of each treatment with five last instar larvae or pupae were used for the laboratory tests. The mortality percentage was determined daily for a week.

6- Field application and evaluation:

Thirty infested field plots by *P. rapae* were treated randomly, 3/biocontrol agent and three plots for untreated control. Each plot contained three lines (2.7 m wide) and 12 m long in total 32.4 m^2 containing 60 plants/plot. The concentration of nematode suspension for field experiment was 2000 IJs/ml with 5% of super film. All field experiments were carried out before sunset in November, 2006 at El-Manawate village, Giza Government. In these tests, all materials were applied in the field as spraying using 10 liters portable spraying on naturally-infested cauliflower plants with *P. rapae*. The portable spraying was washed carefully using water and detergent after each bio-agent

application. To evaluate the performance and efficacy of the tested bio-agent, ten plants from each plot were inspected for bio-agents activity by recording the live stages (larvae and pupa) on the plants in the early morning. The insect counts were done for all treated field plots and the control ones directly before and one week after treatments to resolve the population reduction of *P. rapae* by using Henderson and Tilton formula (1955).

7- Statistical analysis :

The data of the percentage values in this study were normalized using arcsine transformation. The significance of the main effects was determined by analysis of variation (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P < 0.05$) (SAS institute, 1988). The population reduction of *P. rapae* was determined by using Henderson and Tilton formula (1955).

RESULTS AND DISCUSSION

1- Laboratory experiment

The general effect of the of each individual treatment as well as the interactions between treatments on the insect mortality were analyses in Table (1). It may be clear that significant differences occurred between the larvae and pupae of *P. rapae* and the entomopathogenic nematodes isolates and/or strains (B20, H 44, At 4, and At s) and nematode concentrations ($P < 0.05$) at insect instars over three interactions (nematodes strains, nematodes concentration, and insect instars) at ($P < 0.05$) were found. Significant different in the mortality of insect instars of *P. rapae* between the entomopathogenic fungi strains (F 1, F 2, and F3) and fungi concentration ($P < 0.05$) at over three factor interactions (fungi strains, fungi concentrations, and insect instars) at ($P < 0.05$) were found. Finally, Significant different in the mortality of insect instars of *P. rapae* between the two synthetic pesticides (Proclaim & Spinosad) at ($P < 0.05$) and concentration at insect instars over three factors interaction (biopesticides t nematodes type, concentration, and insect instars) at ($P < 0.05$) were found. Mortality required to different used biopesticides simultaneously with host instars was reported in figure (1).

The results clearly show that the virulence of entomopathogenic nematodes is strongly affected by interactions between nematode species and host instars, the virulence of the nematode species relative to each other differed greatly among associated symbiosis bacteria (Dunphy and Webster, 1988). With the synthetic biopesticides Spinosad was high effect more than the Proclaim because its effect work on neural system while proclaim work with digestive system (Hill and Foster, 2003). Different in entomopathogenic fungi pathogenicity required the effect of enzyme mechanisms during the fungi spores penetration and germination (Goettel, et al., 2005).

2- Field experiment

The integrated use of biopesticides (EPN, EPF), and synthetic pesticides with other control agents were investigation, as this could improve resistance management strategies and systemat environmental side-effects caused by chemicals insecticides. For example, brassica crops may be sprayed between five and nine times in a growing season, but the replacement of early-season insecticide sprays with *B. bassiana* to control beetle and caterpillar pests can be effective and economical (Poprawski et al. 1997; Vandenberg et al. 1998). Non-target insects, such as insect predators and parasitic wasps, could be affected by *B. bassiana* but environmental assessment studies in cotton showed this to be a low and acceptable risk (Jaronski et al. 1998).

The results of these studies indicate that spinosad provided a therapeutic and residual level of control against cabbage white butterfly "*P. rapae*". This was equivalent or superior to other biopesticides. The spinosad was equivalent with *Steinernema* spp. (At 4), while both were superior to other biopesticides (Fig. 2). Meanwhile, proclaim and *Heterorhabditis* spp. (B 20) came at the second grade of causing population reduction (Henderson and Tilton, 1955) of *P. rapae* under field conditions (Fig. 2). Moderate insect population reduction was obtained from the use of *B. bassiana* (F 2), while the lower reduction was obtained from the use of *B. bassiana* (F 1) (Fig 2). Some new insecticides, such as Spinosyns (spinosad) and emamectin benzoate (Proclaim) have been shown to be relatively safe on predacious hemipterans, mites, coccinellids, lacewings and some parasitoids (Elzen, 2001). However, the present results showed that all biopesticides, at their field rate concentrations, resulted in a significant variation in general effect of each individual treatment as well as the interaction between thesetreatments (different biopesticides & time) on population reduction of host (Table 2) under field conditions.

The results also indicate that spinosad was the fastest killer of the insect host, followed by proclaim, while the entomopathogenic nematodes came next with regard to host mortality. Finally entomopathogenic fungi showed the least effect. Spinosad acts quickly and has speed of kill comparable to most synthetic insecticides. It acts significantly faster than the other pathogen like *Steinernema* sp., *Heterorhabditis* sp. and *Beauveria* fungi, and other synthetic pesticides. This is largely due to its contact mode of entry (Salgado, 1998), rather than a strict reliance on ingestion as with most traditional synthetic pesticides. Because of the combination of contact and ingestion activity, the onset of insect control occurs quickly and is irreversible. Symptoms appear almost immediately and complete mortality occurs within 24 hours (Salgado, 1998). Meanwhile, the proclaim insecticide controls the larval stages (worms/caterpillars) of certain lepidopterous species. Proclaim insecticide also has some contact activity (Hill and Foster, 2003) to

most larvae but they should ingest it. Shortly after exposure to proclaim insecticide, the affected larvae are paralyzed, stop feeding and subsequently die after 2-4 days. Meanwhile, the entomopathogenic nematodes induce its effect within 3-5 days. The entomopathogenic fungi showed its effect within 5-7 days.

The biological control methods are safe in application and their effects appear slowly and effectively in the environment for example entomopathogenic nematodes once applied to the cauliflower crops, the nematodes locate their insect host by detecting movement and following CO₂ emission. They enter the pest larvae via opening such as the mouth, anus or breathing spiracles, or hack their way directly through weak spots in hosts outer covering. Meanwhile the produced fungal spores or conidia are generally responsible for infection and are dispersed throughout the environment in which the insect hosts are present. When the conidia land on the cuticle of a suitable host, they attach and germinate, initiating cascades of recognition and enzyme activation reactions both by the host and the fungal

parasite (Samson et al. 1988). Invasion of the insect body and circulatory system (haemolymph) occurs once the fungus has passed through the cuticle of the external insect skeleton. Structures and processes for the invasion of insect tissues are similar to plant pathogens, including the formation of germ tubes, appressoria and penetration pegs (Samson et al. 1988). The synthetic pesticides Salgado (1998), spinosyn (Spinosad) and Proclaim with poisoning symptoms were described in a variety of insects, and it was concluded that the first symptoms were due to involuntary muscle contractions caused by excitation of the central nervous system.

So these synthetic chemicals are very fast (kill the host within 1-2 days) and have a residual effect for several days. This will lead to the possibility of using these synthetic biopesticides with alternation of other pathogens to release a highly effective control. This concentrated summation of data may aid in establishing an efficient control strategy capable of minimizing insect population densities below economic thresholds.

Table 1 : Analysis of variance of the effect of different biopesticides agents on *P. rapae* in the laboratory.

Treatments	Df @	Sum of squares	Mean of squares	F value
Nematode strains (NS)	3	0.375	0.125	1.000 *
Nematode concentrations (NC)	2	345.861	172.931	1383.444 ***
NS x NC	6	0.250	0.042	0.333 *
Insect (II)	1	3.125	3.125	25.000 **
NS x II	3	0.375	0.125	1.000 *
NC x II	2	1.750	0.875	7.000 **
NS x NC x II	6	0.583	0.097	0.778 *
Error	48	6.000	0.125	
Fungi strains (FS)	2	0.407	0.352	2.714 **
Fungi concentration (FC)	2	259.593	129.796	1001.285 ***
FS x FC	4	0.407	0.102	0.786 *
Insect (II)	1	0.463	0.463	3.571 **
FS x II	2	0.037	0.019	0.143 *
FC x II	2	0.481	0.241	1.857 **
FS x FC x II	4	0.185	0.046	0.357 *
Error	36	4.667	0.130	
Synthetic pesticides (SP)	1	0.375	0.375	4.50 **
Pesticides concentration (PC)	1	92.042	92.042	1104.50 ***
SP x PC	1	0.375	0.375	4.50 **
Insect (II)	1	3.375	3.375	40.50 ***
SP x II	1	0.042	0.042	0.50 *
PC x II	1	3.375	3.375	40.50 ***
SP x PC x II	1	0.042	0.042	0.50 *
Error	16	1.333	0.083	

@ Df = degree of freedom.

*, **, *** = refer to level of significance (p = 0.05) and variations with different signs within the same group.

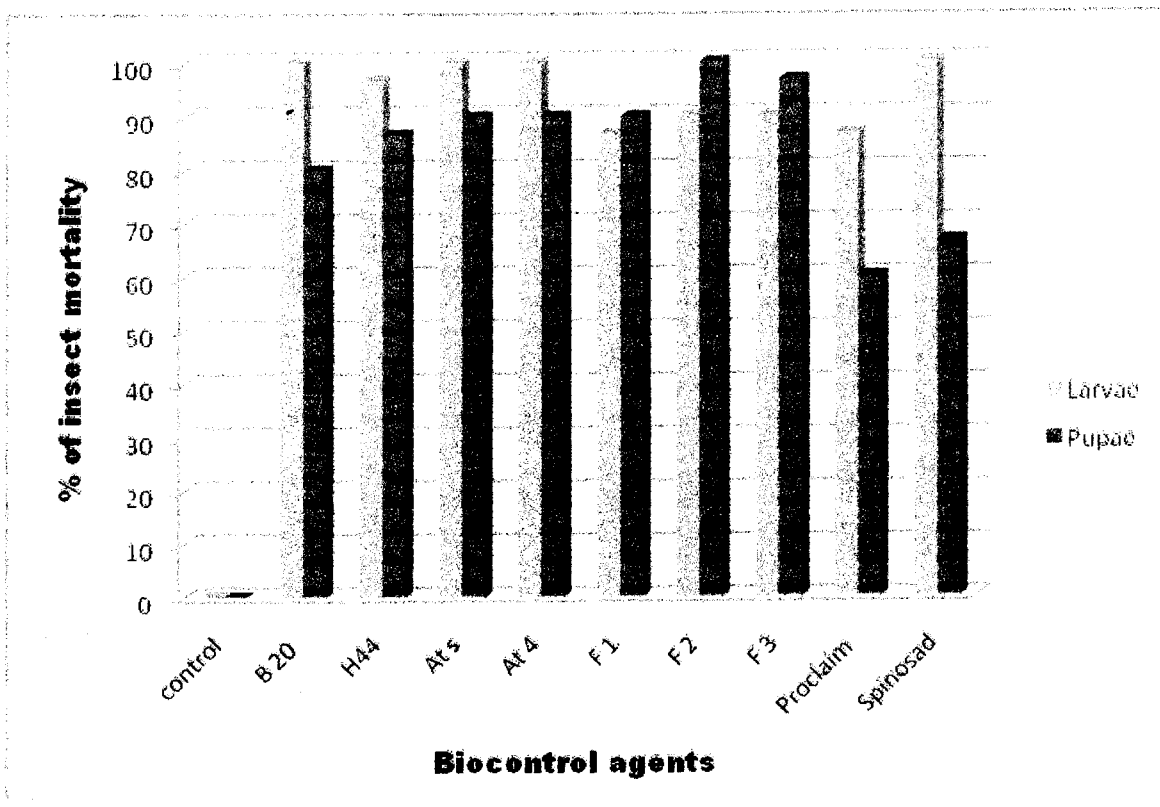


Figure 1. Effect of different biopesticides on larval and pupal stages of *P. rapae*.

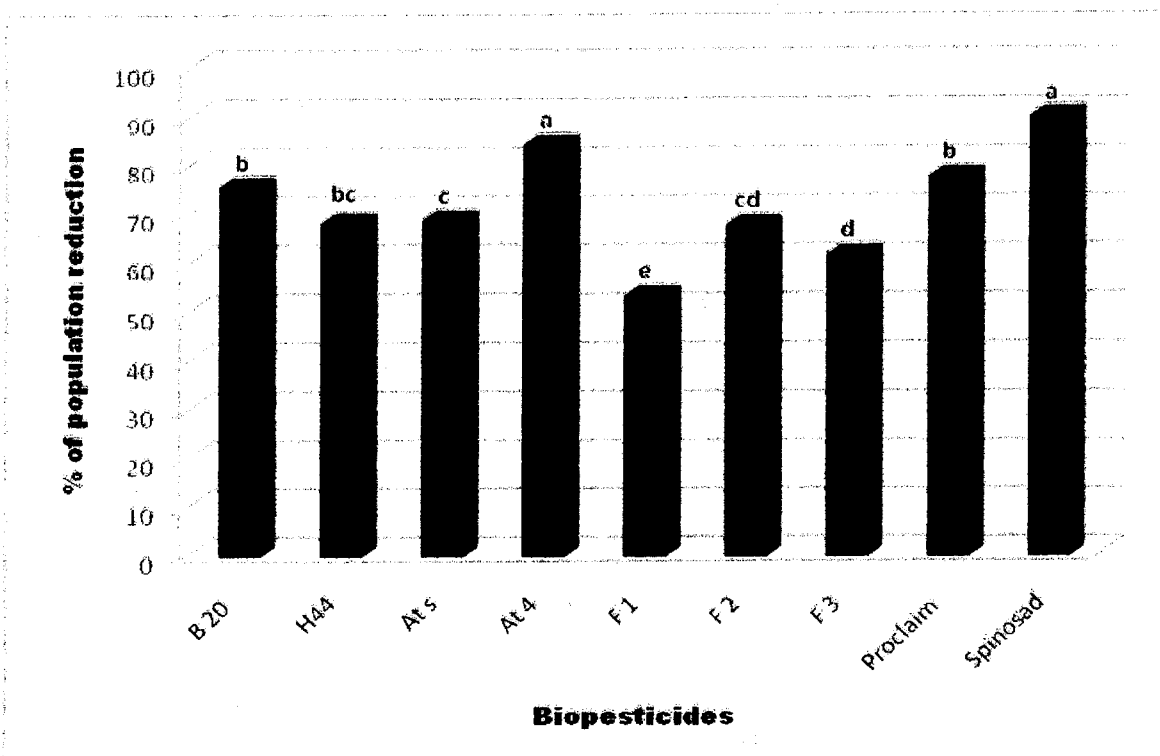


Figure 2. The effect of different biopesticides on population reduction of *P. rapae* under field conditions.

Table 2 : Analysis of variance data showing the effects of different biopesticides on *P. rapae* under field conditions.

Treatments	Df @	Sum of squares	Mean of squares	F value
Plot replication	2	0.196	0.098	0.587 *
Biopesticides (DB)	9	8.117	0.902	5.403 **
Observation time (OT)	1	122.694	122.694	734.926 ***
DB x OT	9	18.793	2.088	12.507 **
Error	38	6.344	0.167	
Total	59	156.144		

@ Df = degree of freedom.

*, **, *** = level of significance (p = 0.05).

ACKNOWLEDGMENTS

The authors appreciate the technical assistance of Prof. Dr. Mohamed Helmi Belal (Professor of Pesticides, Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University), for providing the synthetic biopesticides (Proclaim and Spinosad) and Mr. Khaled Ibn Elwaleed Mahmoud, Applied Center for Entomonematodes, Cairo University, for helping in producing entomopathogenic fungi for field application.

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الملخص العربي

تأثير المبيدات الحيوية المختلفة علي أبو دقيق الكرنب 'بيرس رابا' في حقول القرنبيط

عطوة أحمد عطوة ، بدرالصباح فتوح ، جهاد موسى

قسم بحوث أفات الخضر والنباتات الطبية والعطرية - معهد بحوث وقاية النباتات - مركز البحوث الزراعية
وزارة الزراعة - ٧ ش نادي الصيد - الدقي - جيزة - مصر .

تسبب حشرة أبو دقيق الكرنب (بيرس رابا) والتي تتبع عائلة بيريدي من رتبة حرشفية الأجنحة العديد من الأضرار لمحاصيل الخضر الورقية ومنها علي سبيل المثال القرنبيط ، وتعتبر النيماطودا الممرضة للحشرات والفطريات الممرضة للحشرات والمبيدات المخلقة (البروكلام والإسبينوساد) من المبيدات الحيوية التي تستخدم في الزراعة العضوية أو الزراعة المستدامة ، ولقد كان التأثير الأساسي لهذه المجموعة المستخدمة من المبيدات الحيوية ذو تأثير معنوي وبدرجات مختلفة علي اليرقات والعذارى لحشرة الاختبار (أبو دقيق الكرنب) ، حيث كان تأثير النيماطودا الممرضة للحشرات والتابعة لجنس هيتيرورابديس (العزلة بي ٢٠ والعزلة إتش ٤٤) وكذلك النيماطودا التابعة لجنس شتينرنيما (العزلة آتي إس والعزلة آتي ٤) متقارب وغير معنوي علي طور اليرقة ، في حين كان هناك تأثير معنوي كبير لصالح العزلة (آتي ٤) علي طور العذراء تحت الظروف المعملية . أما بالنسبة للفطريات الممرضة للحشرات والتي تتبع النوع بوفريا باسيانا (العزلة إف ١ ، إف ٢ ، إف ٣) فكانت العزلات الثلاث متوسطة التأثير علي طور اليرقة في حين كانت ذات تأثير معنوي علي طور العذراء وكانت العزلة (إف ٢) هي الأكثر تأثيراً يليها العزلة (إف ٣ ثم إف ١) علي حشرة الاختبار ، بينما كانت اليرقات أكثر حساسية من العذارى للمبيدات المخلقة (البروكلام والإسبينوساد) وكان الإسبينوساد أكثر تأثيراً من البروكلام . وتحت ظروف الاستخدام الحقلية كان الإسبينوساد ذو تأثير واضح ومتبقي لمكافحة الحشرة (أبو دقيق الكرنب) حقلياً وكان له تأثيراً حقلياً في خفض تعداد الآفة مساوي تقريباً للنوع النيماطودي (آتي ٤) بينما كان للإسبينوساد تأثير متبقي علي النبات لعدة أيام (وضح من خلال موت الحشرات) ، وكان لكلاهما (آتي ٤ ، سبينوساد) تأثير أعلى من باقي المبيدات الحيوية المستخدمة في خفض تعداد الآفة حقلياً ، بينما كان البروكلام والسلاطة النيماطودية (بي ٢٠) في المرتبة الثانية للخفض في تعداد الآفة حقلياً ، وكان لسلاطة الفطر (إف ٢) تأثيراً متوسطاً في خفض تعداد الآفة وكان أقل تأثيراً للخفض في تعداد الآفة حقلياً نتج عن سلاطة الفطر (إف ١) . وفي هذه الدراسة سوف نستخدم العديد من المبيدات الحيوية المختلفة لمكافحة حشرة أبي دقيق الكرنب في حقول القرنبيط ، وسوف يقود هذا الاتجاه لإمكانية استخدام المبيدات المخلقة (البروكلام والإسبينوساد) بالتبادل مع الممرضات الحشرية الأخرى (الفطريات والنيماطودا) للحصول علي مكافحة عالية التأثير والتي تؤدي نتيجة استخدام هذه الإستراتيجيات إلي خفض تعداد الآفة الي دون الحد الإقتصادي الحرج.