EFFECT OF MANY PESTICIDES ON VIRULENCE OF TWO ENTOMOPATHOGENIC NEMATODES SPECIES

IBRAHIM S. IBRAHIM
Plant Protection (Pesticides) Dept. Fac. Agric. (Cairo), Al-Azhar Univ., Egypt
Email: i.said@azhar.edu.eg

Abstract: Entomopathogenic nematodes (EPNs) consider one of the most important biological control agents. EPNs have virulence against various insects. Pesticides may be reducing the virulence of EPNs. The aim of this work is studying the effect of some pesticides on the virulence of EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* under laboratory conditions. Pesticides are fungicide mancozeb, insecticide imidacloprid, nematicide cadusafos and herbicide glyphosate isopropylammonium. These pesticides were used at recommended rates of field application. The results showed that *S. carpocapsae* exposed to different tested pesticides was more virulence to third instar larvae of *Spodoptera littoralis* than pesticides-exposed *H. bacteriophora*. The results obtained from this study revealed that most of the tested pesticides did not reduce the virulence of both tested nematodes. Thus, based on these findings, EPN *S. carpocapsae* and *H. bacteriophora* can be used simultaneously with the tested pesticides. Thereby, *S. carpocapsae* and *H. bacteriophora* are suitable to be used in integrated pest management (IPM) programs.

Keywords: Pesticides, *Steinernema carpocapsae, Heterorhabditis bacteriophora*, virulence

1.INTRODUCTION

Entomopathogenic nematodes (EPNs) consider one of the most important biological control agents and commercially available. They have control potential of a wide range of economically important insect pests, especially on soil-dwelling insects, including lepidopterans, coleopterans and dipterans **(Grewal *et al*. 2005 , Susurluk *et al.*, 2011** and **Ulu *et al.*, 2014)**.

The EPNs (Steinernematidae and Heterorhabditidae) are parasites of insects and kill their hosts during 24-48h with inter bacteria carried in the nematode's alimentary canal; steinernematids carry *Xenorhabdus* spp., whereas heterorhabditids carry *Photorhabdus* spp. **(Burnell and Stock 2000; Adams** and **Nguyen, 2002)**.

The life cycle of heterorhabditidae and steinernematidae nematodes involves free-living infective third-stage juveniles (IJs) **(Akhurst, 1986)**. The IJs of both nematodes commonly seek out and enter the insect host through natural openings such as the spiracles, mouth and anus. After the IJs penetrate into the host's hemocoel, the nematode releases the bacteria that propagate and cause fatal septicemia. The bacteria digest the contents of the cadaver and the nematode feeds on the bacterial culture. The nematodes pass through 2 or 3 generations before they produce new infective juveniles (IJs) that emerge from the depleted host cadaver into the soil within two to three weeks **(Hominick *et al*., 1995** and **Downes and Griffen, 1996)**.

Recently, EPNs are widely used in crop-protection strategies and are therefore likely to come into contact with chemical pesticides. It is often desirable to know whether a pesticide can be tank-mixed or applied simultaneously with another pesticide to save time and money, therefore compatibility with integrated pest management **(Grewal, 2002)**. Additionally, EPNs in natural environment are exposed to many of pesticides may be reduce their infectivity **(Atwa *et al.*, 2013** and **korrat, 2018)**.

Most previous studies focused on effect of pesticides on EPNs survival, but little focused on virulence. So, the aim of this work is to evaluate the effect of many of pesticides on the virulence of entomopathogenic nematode *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to insect pests.

2.MATERIALS AND METHODS

The present study was carried out in the laboratory of Plant Protection Department, Faculty of Agriculture, Cairo, AL-Azhar University, Egypt during 2016.

2.1.Selected Pesticides

Selected pesticides in the study were listed in Table (1). Stock solutions of the pesticides were freshly prepared in distilled water at field-recommended rates.

2.2.Entomopathogenic Nematodes

The entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were obtained from Zoology Department, Faculty of Agriculture, Cairo, Al-Azhar University. These strains were not previously exposed to any pesticides. The nematodes, each species separately propagated in the last instar wax moth, *Galleria mellonella* larvae at room temperature (23±1°C) in darkness using methods described by **Kaya** and **Stock (1997)**. *G. mellonella* larvae infected by the nematodes were placed on White traps **(White, 1927)**, at 25±2°C, 65±5 % R.H. Then, the third stage infective juvenile stage (IJs) emerging from cadavers were harvested within 10-15 days of emergence in sterile distilled water. Each species of nematodes kept separately in aqueous suspension at 10°C and were stored for up to one week before the experiment.

2.3.Effect of Selected Pesticides on Virulence of Tested Nematodes

Petri dish (Ø 9cm) filled with 10 ml of pesticide at recommended concentration and 100 µl of nematode concentrate suspension (about 20000 IJs/mL). Nematodes with only distilled water were used as control. Each

Table (1): Basic data about the selected pesticides

Common name	Trade name, Concentration and Formulation*	Classification	Source	Recommended concentrations / feddan
Mancozeb	Dithane M-45 80% W.P.	fungicide	Dow AgroSciences Co.	2000ppm
Imidacloprid	Pestidor 25% W.P.	insecticide	Bayer CropScience	250ppm
Cadusafos	Ragby 20% C.S.	nematicide	Delta agrochemical Co.	750ppm
Glyphosate isopropylammonium	Rophosate 48% S.L.	herbicide	Agrochem Co.	3000ppm

*W.P: Wettable Powder, C.S: Capsule Suspension, S.L: Soluble Liquid Concentrate

treatment had four replicates. Petri dishes were kept at 24±2 °C in darkness for 24 hours. A sieve, 500-mesh was used to obtain the IJs suspensions of pesticide-exposed nematodes; the nematodes were retained on the top of sieve washed for three times, to remove the rest of the pesticide. The washed IJs collected from the sieve were concentrated in 3 ml of distillated water and used for infected third instar larvae of *Spodoptera littoralis* (as host). Thousand live IJs for *Steinernema carpocapsae* or *Heterorhabditis bacteriophora* in 1 ml of water were applied over filter paper in the bottom of plastic boxes (9cm × 5cm × 4cm). Ten 3rd instar larvae of *S. littoralis* were put into a plastic box with fresh castor bean leaves as a source of food for the larvae. Plastic boxes were kept at 24±2°C in darkness. Untreated (unexposure) nematode suspension was used as control. Each treatment had four replicates. Mortality percentages of *S. littoralis* larvae were determined after 24 and 72 h **Stepanka (2011)**.

3.Results and discussion

3.1.Effect of Selected Pesticides on Virulence of Tested Nematodes

The effect of four different pesticides at recommended concentration of field application on virulence of EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against *Spodoptera littoralis* 3rd instar larvae was determined under laboratory conditions. The results in Table (2) showed that *S. carpocapsae* and *H. bacteriophora* that exposed to imidacloprid, glyphosate isopropylammonium and mancozeb had the highest infectivity rates against the 3rd instar larvae of *S. littoralis,* there were no difference between these treatments and control, which gave 100% mortality percentages after 72hr of treatment the 3rd instar larvae of *S. littoralis.* However, *H. bacteriophora* that exposed to cadusafos had the lowest infectivity rates against the 3rd instar larvae of *S. littoralis* which gave 50% mortality percentages after 24hr of treatment the 3rd instar larvae of *S. littoralis*. On the other hand, the imidacloprid, glyphosate isopropylammonium and mancozeb treatments had little affected *H. bacteriophora* infectivity whereas the mortality percentages of *S. littoralis* larvae were 90, 95 and 97%, respectively, compared to 100% mortality in the control at 24hr. In general, pesticides-exposed *S. carpocapsae* was higher infectivity than pesticides-exposed *H. bacteriophora* against third instar larvae of *S. littoralis*. The results obtained from this study revealed that imidacloprid, glyphosate isopropylammonium and mancozeb did not effect on the virulence of both tested nematodes. These results are agreed with many researches, **(Rovesti and Deseo, 1991 and Atwa, 1999)** revered to that EPNs in the family's Steinernematidae and Heterorhabditidae were effective after exposure to many chemical pesticides. Additionally, **Gaugler & Campbell (1991)** and **Ishibashi & Takii (1993)** indicated that *S. carpocapsae* was more active in the presence organophosphate pesticides under laboratory conditions. Such chemicals may stimulate inactive nematodes and thereby enhance their infectivity against the target insects. This indicates a potential for combined applications of nematodes with pesticides. Also, **Zang *et al.*, (1994) ,Gordon *et al.* (1996)** and **Atwa *et al.* (2013)** reported that, no toxic effect of many fungicides, insecticides of a variety of carbamates and organophosphates on nematodes infectivity. As well as, **korrat (2018)** found

Table (2): Mortality percentages of *Spodoptera littoralis* third instar larvae caused by *Steinernema carpocapsae and Heterorhabditis bacteriophora* previously exposed to the recommended concentrations of pesticides.

Treatment	%Mortality of *Spodoptera littoralis*			
	Steinernema carpocapsae		*Heterorhabditis bacteriophora*	
	after 24h	after 72h	after 24h	after 72h
Unexposed EPNs*(control)	100	100	100	100
Imidacloprid	97.50	100	90	100
Glyphosate isopropylammonium	97.50	100	95	100
Mancozeb	100	100	97	100
Cadusafos	90	100	50	70

*EPNs: Entomopathogenic nematodes.

that the IJs of *S. carpocapsae* and *H. bacteriophora* that exposed to chlorpyrifos-methyl and lufenuron formulations had the highest infectivity rates against the 4th instar larvae of *S. littoralis* that gave mortality percentages > 80 % after 3 days of treatment to the 4th instar larvae of *S. littoralis.* However, emamectin benzoate and methomyl formulations-exposed IJs of *S. carpocapsae* and *H. bacteriophora* resulted in poor efficacy on *S. littoralis* larvae with mortality percentages not exceed 36.7 %.

The results obtained from this study showed that nematode *S. carpocapsae* which survived the pesticide was more virulence to third instar larvae of *S. littoralis* than *H. bacteriophora*. This means that *S. carpocapsae* was more tolerant to selected pesticides than *H. bacteriophora.* Likewise, results obtained by **Rovesti *et al.* (1988)** who indicated that Steinernematids were more tolerant than Heterorhabditids to many pesticides. Such differed tolerance of EPNs may be attributable to the differences in nematode's acetylcholinesterase concentration **(Shamseldean *et al.*, 2005** and **Atwa, 2013)**. One factor related to the infectivity of nematodes was the lipid amounts present in infective juveniles (IJs) **Wright** and **Perry (2002)**.

Generally, most of the selected pesticides did not effect of virulence of the tested nematodes. Based on these findings, EPN *S. carpocapsae* and *H. bacteriophora* can be used simultaneously with the tested pesticides, therefore, tank-mix application is possible for enhance controlling of *S. littoralis* larvae. Thereby, *S. carpocapsae* and *H. bacteriophora* are suitable to integrated pest management (IPM) programs. Further investigations may need new studies with new pesticides and species or strain.

Finally, results obtained from this study set the start point for further studies on EPN and pesticides interaction which should be taken into account when implementing and assessing success of EPN in pest management programs.

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تأثير العديد من المبيدات على القدرة المرضية لنوعين من النيماتودا الممرضة للحشرات

إبراهيم سعيد إبراهيم

قسم وقاية النبات – كلية الزراعة بالقاهرة – جامعة الأزهر- مصر

الملخص العربى:

تعتبر النيماتودا الممرضة للحشرات واحدة من أهم عوامل المكافحة البيولوجية. فهى لديها قدرة مرضية ضد العديد من الحشرات. والمبيدات ربما تُقلل من تلك القدرة المرضية للنيماتودا الممرضة للحشرات. ولذا فان الهدف من هذا البحث هو دراسة تأثير بعض مبيدات الآفات على القدرة المرضية لنوعين من النيماتودا وهما *Steinernema carpocapsae* و *Heterorhabditis bacteriophora* تحت ظروف المعمل. والمبيدات المستخدمة هى مبيد الفطر mancozeb ، مبيد حشرى imidacluprid ، مبيد النيماتودا cadusafus ومبيد حشائش glyphosate isopropylammonium . وهذة المبيدات أُستخدمت بالمعدل الحقلى الموصى به محسوباً كجزء فى المليون. ولقد أظهرت النتائج أن النوع *S. carpocapsae* الذى تعرض للمبيدات المختلفة كان أكثر قدرة مرضية ليرقات العمر الثالث لدودة ورق القطن *Spodoptera littoralis* من النوع *H. bacteriophora* المُعرض لنفس المبيدات. كما أشارت النتائج إلى أن معظم المبيدات المختارة لم تقلل من القدرة المرضية للنيماتودا. وبناءً على هذه النتائج ، يمكن إستخدام كل من *S. carpocapsae* و *H. bacteriophora* في وقت متزامن مع المبيدات التي تم اختبارها. وبالتالي، فإن *S. carpocapsae* و *H. bacteriophora* ملائمتان للإستخدام في برامج المكافحة المتكاملة للآفات.