# Laboratory tests on the integrated management of cotton leaf worm *Spodoptera littoralis* (Boisd.) by using some insecticides and entomopathogenic nematodes

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**ABSTRACT**

Effects of certain insecticides *i.e.* Coragen, Nomolt, Ekio and Magic smart with two species of entomopathogenic nematodes, *Steinernama carpocapsae* and *Heterorhabdits bacteriophora* were tested against cotton leaf worm *Spodoptera littoralis.* All tested insecticides caused less mortality to the tested entomopathogenic nematodes. Magic Smart significantly surpassed other insecticides causing the mortality of 11.6 and 13.8 % to the entomopathogenic nematodes *S. carpocapsae* and *H. bacteriophora* opposite to Coragen that caused slight mortality, 2.5 and 4.4%, respectively and didn't differ significantly than the check. Nomolt and Ekio gave mortality of 5.4 and 8.8 % to *S. carpocapsae* as well as 8.8 and 12.0 % to *H. bacteriophora* respectively. These results show that the used insecticides have less effect on entomopathogenic nematodes specially *S. carpocapsae*. The entomopathogenic nematode *S. carpocapsae* exposed to Coragen, Nomolt, Ekio and Magic smart gave 92.5, 85.0, 80.0 and75.0 % mortality to cotton leaf worms 5th larval instar, respectively. The entomopathogenic nematode *H. bacteriophora* exposed to Coragen ,Nomolt, Ekio and Magic smart gave 83.4, 77.2, 71.0 and 60.6 % mortality % of *S. littoralis*, respectively. Joint effect of insecticides and entomopathogenic nematodes show that the mixture between *S. carpocapsae* with each of Coragen, Nomolt, Ekio and Magic smart at the quarter lethal concentration ($LC_{25s}$) per each gave synergism, synergism, additive and additive effects, respectively. As while *H. bacteriophora* mixed at the $LC_{25}$ with the $LC_{25s}$ of Coragen, Nomolt, Ekio and Magic Smart attained synergism, additive, additive and additive effects, respectively.

**Key words**: Entomopathogenic nematodes, Co-toxicity, S.carpocapsae, H. bacteriophora

## Introduction

Combination of biocides and chemical pesticides may be taken in consideration with any prospected integrated pest management program. The used combined factors in the IPM program must be chosen to sustain each other against the target pest with minimum effect on the other non -target organisms. In this regard synergistic or antagonistic effects between the entomopathogenic nematodes as biocides and pesticides used in combination had been studied by Rao *et al*. (1975), Hara and Kaya (1983), Qin (1984), Das and Divakar (1987), Ishibashi *et al*. (1987), Kaya and Burlando (1989), Rovesti *et al*. (1990), El-Kifl and Sammour (1991), Gaugler and Campell (1991), Hassan (1993), Mourad *et al*. (1994), Saleh and Sammour (1995), Peters. and Poullot (2004) Negrisoli Jr. *et al.* (2010) and Hassan (2016).

The present study was carried out to evaluate the combined effects of two entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* and four insecticides, Corag*en*, Nomolt, Ekio and Magic Smart against cotton leaf worm, *Spodoptera littoralis* (Boisd.).

## Materials and Methods

### *The tested insects and entompathogenic nematodes:-*

Insect rearing and entomopathogenic nematodes multiplication, as well as bioassays, were performed at Pesticide Researches Laboratory, Plant Protection Department, Faculty of Agricultural, Minia University

Greater wax moth larvae were produced in incubator at 28± 2 ºC, 60% relative humidity and 12 h photoperiod, according methodology modified by Parra (1998). Cotton leaf worms *Spodoptera littoralis* was reared in Plant Protection Department Laboratory, Faculty of Agriculture Minia University on castor leaves for several generations according to the method explained by Entompathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were obtained from Laboratory of Nematology, Faculty of Agriculture Cairo University and reared on *Galleria mellonella* for several generations in Zoology Research Laboratory, Plant Protection Department, Faculty of Agricultural, Minia University according to Kaya and Stock (1997). Infective juveniles of nematodes stored in incubator at 15 ºC in plastic bottles arranged horizontally according to Hassan (2016)

### The tested insecticides:-

The tested insecticides were Coragen (chlorantaniliprole) this insecticide stimulates the release of calcium in muscle cell cytoplasm causing the paralysis of muscles within 3-04 days after treatment. Other insecticides, Nomolt (teflubenzuron), Ekio (novaluron) and Magic Smart (lufenuron), act as chitin synthesis inhibitors preventing the formation of chitin in the new cuticle, make the new cuticle unable to persist the pressure of body fluids and muscle contractions during the molting process, so they called anti-molting compounds.

**The effect of mixing entomopathogenic nematodes with insecticides:-**

Entomopathogenic nematodes at the concentration of 2500 IJs / ml (the field rate) with the tested insecticides with their field usage rate (Coragen 20% SC at 60 ml / 400 L., Nomolt 15% SC 50ml / 100 L., Ekio 10% EC 60 ml/ 100 L. and Magic Smart 5% EC at 160 ml / 400 L.),) was applied for evaluating IJs viability and infectivity after being exposed to insecticides. According to methodology suggested by Negrisoli Jr. *et al* (2010)

One liter of each insecticide formulation was prepared proportionally to the double concentrations that would be normally applied in the field. From these solutions, 1 mL aliquots of each insecticide prepared solution were placed in glass test tube distilled and latter 2500 IJs per each nematode species contained in 1 mL of distilled water were added to the mentioned above tubes. Each tube represented one replicate from five. These tubes were held in incubator at 22 ±1 C; RH of 70±10% with 12 h photoperiod.

Nematode mortality was evaluated 48 h after their exposure to the insecticide. Thus 0.1 ml from the suspension of each tube was removed to watch class and 100 IJs were observed under the stereo microscope, considering dead those that don't reacted when touched with a probe. Mortality percentages were corrected according to Abbot' formula, Abbot (1925)

Nematode infectivity test were carried by filling the tubes of the previous test with 3 ml of distilled water and placed to rest for 30 min in incubator at 15 C. Supernatant liquid (approximately 3 ml) was then withdrawn and rinsing process repeated for three times. After the last rinsing, a volume of 0.2 mL (approximately 400 IJs) were drawn from the bottom of each tube and distributed in five Petri dishes (9 cm diameter) containing filter paper previously wetted with 1.8 ml distilled water for each treatment with each Petry dish as one replicate. Each dish received ten fourth instar larvae of *S. littoralis*, kept in incubator at 22 ± 1ºC and R.H. of 70 ±10 % with 12 h photoperiod for five days. Dead larvae were counted, transferred to Petri dishes (9 cm diameter) containing dry filter paper and maintained into darkness for more three days. Finally they were dissected to verify nematode' presence (Negrisoli Jr. *et al.*, 2010). The experiment's statistical design was completely random.

**Data analysis:**

Nematodes and cotton leaf worm mortality data were subjected to variance analysis and differences between treatment means were estimated by Chi square test at 0.05 probability. The effect of the treatments on nematodes infectivity over *S. littoralis* larvae was classified according Peters and Poullot (2004), based on IOBC guideline

E%=1-(It/ Ic) x 100

It= mortality in treatment

Ic= mortality in control treatment

Classification of insecticides effect on nematodes infectivity

1=non effect (E<30 %) 2=slightly effect (E=30-79%) 3=moderately effect E >79

**Combined effect of nematodes and insecticides:-**

To determine the combined effect of nematodes and insecticides mixtures of insecticides and nematodes at the concentration of LC 25 for each were applied against *S. littoralis* 5$^{th}$ larval instar and mortality percentages were estimated

Estimation of LC25 of nematodes was by testing seven concentrations of 2000, 5000, 10000, 20000, 30000, 40000 and 50000 infective juveniles (IJs) of each *H. bacteriophora* and *S. carpocaopsae* nematodes / liter were prepared in distilled water. One ml of nematode suspension of each concentration was dispersed on five larvae of *S. littolaris* (5$^{th}$ instar) supplied with piece of castor leaf in Petri dishes 15 cm in diameter.

As for insecticides different concentrations of each insecticide were tested. Mortality of larvae by nematodes or insecticides was corrected by Abbot's formula (1925). LC 25s were estimated according to Finney (1971).

Mixtures of insecticides and nematodes at the concentration of $LC_{25}$ for each were prepared. One ml of each mixture was applied on larvae supplied with castor leaf in Petri dishes. Each treatment was replicated four times. Average of mortality was estimated and corrected with control treatment. Co-toxicity factor (C.F.) was determined according to Mansour *et al* (1966) as follows: (Observed mortality - Expected mortality / % Expected mortality) × 100.

A positive C.F. value of 20 or more was considered synergism. Negative value of 20 or more was considered antagonism. Any value lies between -20 and + 20 was taken as an additive effect

**Results and Discussion**

Data in Table 1 show that Magic was the insecticide that significantly surpassed others causing the highest mortality 11.6 % to *Steinernema carpocapsae* opposite to Coragen that caused less mortality to this nematode and didn't differ significantly than the check. Nomolt and Ekio gave mortality of 5.4 and 8.8 % to *S. carpocapsae*, respectively. These results show that the used insecticides specially Coragen have less effect on entomopathogenic nematodes specially *S. carpocapsae*. Entomopathogenic nematodes are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes including chemical pesticides, surfactants, fertilizers and soil amendments. Often it is desirable to tank mix one or more inputs to save time and money. Infective juveniles are tolerant of short exposures (2-6 h) to most agrochemicals including herbicides, fungicides,

acaricides and insecticides (Rovesti and Deseo, 1990; Ishibashi and Takii, 1993) and therefore, can often be tank-mixed.

Steinernematid and heterorhabditid nematodes can survive exposure to many chemical pesticides (Hara and Kaya, 1982; Rovesti and Deseo, 1990; Rovesti *et al.*, 1989). However, infective juveniles are highly susceptible to several nematicides likely to be found in the agroecosystem (Rovesti and Deseo, 1990, 1991).

Glazer *et al*. (1997) examined genetic selection as a means of enhancing resistance of *H. bacteriophora* strain HP88 to the nematicides: fenamiphos (organophosphate), oxamyl (carbamate) and averrmectin (biologically- derived product). After 11 rounds of selection, resistance to the nematicides as well as several traits relevant to bio-control efficacy including virulence, heat tolerance, and reproduction potential were examined

Data tabulated in Table 2 display the mortality percentages of *Spodoptera littoralis* treated by *S. carpocapsae* previously mixed with different insecticides i.e. Coragen, Nomolt, Ekio or Magic.

The entomopathogenic nematode *S. carpocapsae* exposed to Coragen gave high mortality to *S. littoralis* (92.5%) and didn't significantly differ than check treatment (*S. carpocapsae* unexposed to insecticides) that caused 95% mortality to *S. littoralis* and the treatment effect (E, value) was 2.6 that mean non effect of this insecticide on *S. carpocapsae* infectivity. This nematodes exposed to Nomolt gave 85% mortality and E, value 10.5. Ekio caused moderated loss of infectivity of *S. carpocapsae* (80% mortality of cotton leaf worms) when compared with control (95% mortality of cotton leaf worms). On the other hand nematodes exposed to Magic smart significantly differed with other insecticides and check treatment that caused less mortality 75% to *S. littoralis* and treatment effect value was 23.7 that still in the range of non-effect on nematode infectivity.

Rovesti and Deseo (1990) observed that Chitin synthesis inhibitors didn't affected *S. carpocapsae* viability. These insecticides didn't cause noticeable inhibition in reproduction and development of *S. carpocapsae* (Hara and kaya, 1982, 1983)

Data in Table 3 show that Magic Smart as was the insecticide that significantly surpassed others causing the highest mortality of *S. carpocapsae* it also gave highest mortality 13.8 % to *Heterorhabditis bacteriophora* opposite to Coragen that caused less mortality to this nematode (4.4%) and didn't differ significantly than the check. Nomolt and Ekio gave mortality of 8.8 and 12.0 % to *H. bacteriophora*, respectively. On the other hand these results show that *H. bacteriophora* tend to be more sensitive to the tested insecticides than *S. carpocapsae*. Gaugler 2002 mentioned that heterorhabditis are more sensitive to chemical challenges, including pesticides, than steinernematids.

Table 4 explains that the entomopathogenic nematode *H. bacteriophora* exposed to Coragen gave high mortality to *S. littoralis* (83.4%) but significantly was low than the check (96.0%) and the estimated treatment effect of insecticide on nematode infectivity (E, value) was 13.1 (non-effect). This nematode exposed to Nomolt gave 77.2 % mortality of *S. littoralis* and E value 19.6 (Non-effect). Ekio caused moderated loss of infectivity of *H. bacteriophora* (71.0% mortality of *S. littoralis*) with E, value of 26.0 (non-effect on nematode infectivity). On the other hand *H. bacteriophora* exposed to Magic smart significantly caused less mortality 60.6 % to *S. littoralis*. The estimated treatment effect of Magic smart on *H. bacteriophora* infectivity on *S. littoralis* was 37.5 that mean slightly effect of Magic smart on *H. bacteriophora* infectivity.

**Table 5** explains propit data established from toxicity line equations of tested entomopathogenic nematodes on *S. littoralis* 5$^{th}$ larval instar the estimated LC50s of Coragen, Nomolt, Ekio and Magic smart were 234.72, 3.88, 19.84 and 13.98 mg / l. while the $LC_{25}$ values were 11.84, 0.61, 5.00 and 3.91 mg / l. for these insecticides, respectively. On the other hand the estimated $LC_{50s}$ of *S.carpocapsae* and *H. bacteriophora* were 27531 and 19780 infective juveniles per liter (IJs), while the $LC_{25s}$ for these nematodes were 6003 and 4343 IJs, respectively

Data in **Table 6** show the co-toxicity of the tested insecticides and entomopathogenic nematodes. The mixture of *S. carpocapsae* at the LC25 with the LC25 of Coragen, Nomolt, Ekio or Magic Smart attained co-toxicity coefficients +30, +20, +10 and -10, respectively. These results mean that the effects of these mixtures gave synergism, synergism, additive and additive actions, respectively. On the other hand the mixture of *H. bacteriophora* at LC25 with the LC25 of Coragen, Nomolt, Ekio or Magic Smart recorded co-toxicity coefficients +20, +10, -10 and -16, respectively. So these trial effects were synergism, additive, additive and additive, respectively.

**Table 1.** Effect of certain insecticides on the mortality of *Steinernema carpocapsae* at 22± 1ºC and RH 70 ± 10%

| Treatment | Mortality % |
|---|---|
| Control (only distilled water) | 0.0 c |
| Coragen | 2.5 c |
| Nomolt | 5.4 abc |
| Ekio | 8.8 ab |
| Magic Smart | 11.6 a |

1-Mortality percentages were corrected according to Abbot's formula
2-Mortalities followed by the same litters didn't significantly differ according to Chi square test.

**Table 2.** Effect of certain insecticides on the infectivity (average ±SE) of *Steinernema carpocapsae* measured by *Spodoptera littoralis* 5th larval instar mortality at 22± 1ºC and RH 70 ± 10%

| Treatment | % mortality of cotton leaf worms | Treatment effect (E%) | Effect classification |
|---|---|---|---|
| Control | 95.0 a | - | - |
| Coragen | 92.5 a | 2.6 | 1 |
| Nomolt | 85.0 b | 10.5 | 1 |
| Ekio | 80.0 bc | 15.8 | 1 |
| Magic | 72.5 c | 23.7 | 1 |

1- Mortalities followed by the same litters didn't significantly differ according to Chi square test.
2-E%=1-(It/ Ic) x 100 It= mortality in treatment Ic= mortality in control treatment
Classification of insecticides effect on nematodes infectivity
1=non effect (E<30 %) 2=slightly effect (E=30-79%) 3=moderately effect E >79

**Table 3.** Effect of certain insecticides on the mortality of *Heterorhabditis bacteriophora* at 22± 1ºC and RH 70 ± 10%

| Treatment | Mortality % |
|---|---|
| Control (Nematodes didn't exposed to insecticides) only distilled water) | 0.0* c |
| Nematodes exposed to Coragen | 4.4 bc |
| Nematodes exposed to Nomolt | 8.8 abc |
| Nematodes exposed to Ekio | 12.0 ab |
| Nematodes exposed to Magic | 13.8 a |

1-Mortality percentages were corrected according to Abbot's formula
2-Mortalities followed by the same litters not significantly differed according to Chi square test.

**Table 4.** Effect of certain insecticides on the infectivity of *Heterorhabditis bacteriophora* measured by *Spodoptera littoralis* 4th larval instar mortality at 22± 1ºC and RH 70 ± 10%

| Treatment | % mortality | Treatment effect (E%) | Toxicity classification |
|---|---|---|---|
| Control | 96.0 | - | - |
| Coragen | 83.4 | 13.1 | 1 |
| Nomolt | 77.2 | 19.6 | 1 |
| Ekio | 71.0 | 26.0 | 1 |
| Magic | 60.6 | 37.5 | 2 |

1-Mortalities followed by the same litters didn't significantly differ according to Chi square test.
2-E%=1-(It/ Ic) x 100
It= mortality in treatment
Ic= mortality in control treatment
Classification of insecticides effect on nematodes infectivity
1=non effect (E<30 %) 2=slightly effect (E=30-79%) 3=moderately effect E >79

**Table 5.** Probit data established from toxicity line equations of tested insecticides and entompathogenic nematodes on *S. littoralis* 5th larval instar

| Treatments | Toxicity line equation | Slope | D.F. | $LC_{50}$ | $LC_{25}$ |
|---|---|---|---|---|---|
| Coragen (chlorantraniliprole) | Y=2.0946+2.0821 | 2.0946 | 3 | 24.72 mg / l. | 11.84 |
| Nomolt (teflubenzuron) | Y=0.8311X+4.5101 | 0.8311 | 6 | 3.88 mg / l. | 0.61 |
| Ekio (novaluron) | Y=1.1205X+3.546 | 1.1205 | 4 | 19.84 mg / l. | 5.00 |
| Magic smatrt (Lufenuron) | Y=1.2103X+3.6137 | 1.2103 | 7 | 13.98 mg /l. | 3.91 |
| *S. carpocapsae* | Y=1.0129X+0.5029 | 1.0129 | 5 | 27531.0 IJs / l. | 6003.0 IJs / l. |
| *H. bacteriophora* | Y=1.0175X+0.6286 | 1.0175 | 5 | 19780.0 IJs / l. | 4343.0 IJs /l. |

**Table 6.** The combined effect of *Steinernema carpocapsae* mixtures with certain insecticides on *S. litoralis* 5th instar larvae under laboratory conditions

| Mixtures (Nematode + insecticide) | $LC_{25}$ + $LC_{25}$ IJs / liter + mg / l. | Mortality % | | |
|---|---|---|---|---|
| | | Expected | Observed | Co-toxicity coefficient |
| *S. carpocapsae* + Coragen | 6003.0 + 11.84 | 50 | 65 | +30 Synergism |
| *S. carpocapsae* + Nomolt | 6003.0 + 0.61 | 50 | 60 | +20 Synergism |
| S. carpocapsae + Ekio | 6003.0 + 5.00 | 50 | 55 | +10 additive |
| *S. carpocapsae* + Magic smart | 6003.0 + 3.91 | 50 | 45 | -10 additive |
| *H. bacteriophora*+ Coragen | 4343.0 + 11.84 | 50 | 60 | +20 synergism |
| *H. bacteriophora*+ Nomolt | 4343.0 + 0.61 | 50 | 55 | +10 additive |
| *H. bacteriophora*+ Ekio | 4343.0 + 5.00 | 50 | 45 | -10 additive |
| *H. bacteriophora*+ Magic smart | 4343.0 + 3.91 | 50 | 42 | -16 additive |

## Conclusion

Based on the present study the compatibility of the insecticides, Coragen, Nomolt, Ekio and Magic Smart with the entomopathogenic nematodes, *S. carpocapsae* and *H. bacteriophora* at the LC25 for each may be possible and resulting synergism or additive effects than using each alone against cotton leaf worm.

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**إختبارات معملية على المكافحة المتكاملة لدودة ورق القطن بإستخدام بعض المبيدات الحشرية والنيماتودا الممرضة للحشرات**

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قسم وقاية النبات– كلية الزراعة –جامعة المنيا

تم إختبار تأثير المبيدات الحشرية كوراجين، نومولت، إكيو و ماجيك إسمارت مع نوعين من النيماتودا الممرضة للحشرات إشتاينرنيما كاربوكابسا و هيتيرورابديتيس باكتريوفورا ضد دودة ورق القطن العمر اليرقى الخامس. وأوضحت النتائج أن تأثير هذه المبيدات عامة كان بدرجة ضعيفة على النيماتودا المختبرة إلا أن مبيد ماجيك إسمارت كان أكثر تأثيرا على النيماتودا اللمرضة للحشرات عن غيره من المبيدات حيث تسبب فى قتل ١١.٦ ، ١٣.٨ % من نيماتودا إشتاينرنيما كاربوكابسا وهتيرورابديتيس باكتريوفورا مقابل ٢.٥ ، ٤.٤% نسب موت لهذين النوعين من النيماتودا على التوالى عند تعرضهما لمبيد كوراجين، أما مبيدى نومولت و إكيو فكان تأثيرهما القاتل على النيماتود متوسط حيث كانت نسب الموت على التوالى ٥.٤ ، ٨.٨ % للنيماتودا إشتاينرنيما كاربوكابسا و ٨.٨ ، ١٢ % للنيماتودا هيتيرورابديتيس باكتريوفورا هذه النتائج توضح أن النيماتودا إشتاينرنيما كاربوكابسا كانت أقل تأثراً بالمبيدات المستخدمة عن النيماتودا هيتيرورابديتيس باكتريوفوراز

تبين من النتائج أن النيماتودا إشتاينرنيما كاربوكابسا المعرضة للمبيدات الحشرية كوراجين، نومولت، إكيو و ماجيك إسمارت أعطت معملياً نسب قتل ٩٢.٥،٨٥.٠، ٨٠.٠ و ٧٥.٠ % للعمر اليرقى الخامس لدودة ورق القطن أما بالنسبة للنيماتودا هيتيرورابديتيس باكتريوفورا المعرضه لهذه المبيدات فقد أحدثت عدوى وقتل للعمر اليرقى الخامس لدودة ورق القطن بنسب ٨٣.٠، ٧٧.٢، ٧١.٠ و ٦٠.٦ % على التوالى أظهر التأثير المشترك للمبيدات الحشرية مع النيماتودا الممرضة للحشرات أنه عند خلط التركيز القاتل ل ٠.٢٥للنيماتودا إشتاينرنيما كاربوكابسا مع التركيزات الربع قاتلة لكل من كوراجين، نومولت، إكيو و ماجيك إسمارت أعطى تأثيرات منشطة ، منشطة ، إضافية و إضافية على التوالى أما فى حالة خلط التركيز الربع قاتل للنيماتودا هيتيرورابديتيس باكتريوفورا مع التركيزات الربع قاتلة لهذه المبيدات فكان التأثير منشط، إضافى ، إضافى و إضافى على التوالى