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# Compatibility of Entomopathogenic Nematode (*Steinernema* sp.) and Coccinelid (*Cheilomenes sexmaculata*) with Insecticides Registered against Fall Army Worm (*Spodoptera frugiperda*) in Corn

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## ABSTRACT

Cyantraniliprole, Chlorantraniliprole, Emamectin benzoate, Spinosad, Flubendiamide, Teflubenzuron, Novaluron+Indoxacarb, Broflanilide, Chlorafenapyr and Azadirachtin are some important registered insecticides against *S. frugiperda* in corn. Bioagents such as entomopathogenic nematodes (EPNs) and lady beetles have also role to reduce its population. Integration of bioagents with chemical insecticides is current need for sustaianable pest management. So, the present evaluation was conducted at laboratory condition to knowhow the fate of EPNs, *Steinernema* sp. and coccinelid (*Cheilomenes sexmaculata)* after their exposure with above mentioned insecticides used for *S. frugiperda*. Lab research was conducted during *spring* (2021) at BCKV, Burdwan campus (West Bengal). EPNs was multiplied on larva of *Corcyra cephalonica. Cheilonemes sexmaculata* (grubs and pupa) was collected from field. Ten novel insecticides recommended for *S. frugiperda* were evaluated against the mentioned bioagents. Corected mortality data of EPNs and coccinelid was analyzed statistically and accordingly insecticides were grouped into different toxicity class. Except Emamectin benzoate, all the chemicals were proved to be safe to *Steinernema* sp. E. benzoate caused 100 % reduction to insect infectivity by EPN, followed by Chlorafenapyr (80 %). Slightly harmful effect for grubs (*C. sexmaculata*) was noted in Cyantraniliprole and Emamectin benzoate and for pupa in Emamectin benzoate and Novaluron + Indoxacarb. Otherwise all others were harmless both for grubs and pupal stage of *C. sexmaculata.* These pieces of information might be practical in decision making for IPM of *S. frugiperda.*

**Keywords** Fall armyworm, EPN, Coccinelid, Corn, Novel insecticides.

## INTRODUCTION

Globally, the Corn is known as the queen of cereals. It has the highest genetic production potential. It is cultivated in around 160 countries covering an area of about 150 m ha. In India, there was a production of 31.51 million tones of corn from an area of 9.9 million hectares during 2020-21 (Anon 2022). This crop is infested by number of insect pests. The most devastative key pest is fall armyworm, *Spodoptera*

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*frugiperda* Smith (Lepidoptera: Noctuidae). It is managed by indiscriminate use of insecticides that invites lesser efficiency along with various serious harms such as 3 R's (residue in food, resistance and resurgence of insects), biotype development in insect, demolition of beneficial agents, illness of human, environment pollution and other depressing outcomes (Diez–Rodriguez and Omoto 2001, Kwizera and Susurluk 2017, Sabry *et al.* 2016).

Some important species of *Spodoptera* ((i.e. *frugiperda, exigua, litura* and *littoralis)* are controlled by bio-agent entomopathogenic nematodes (EPNs). The said EPNs can reproduce inside of these species of *Spodoptera* under laboratory conditions (Fuxa *et al.* 1988, Epsky and Capinera 1993, Park *et al.* 2001, Campos-Herrera and Gutierrez 2008, Garcia *et al.* 2008).

The corn ecosystem includes a diversity of predatory lady beetles such as *Coccinella septumpunctata, Coccinella transversalis, Cheilomenes sexmaculata.* Their high biotic potential and polyphagous predation both by adult and larval stages make them effective bio-agents in management of corn pests. Some essential prey materials are aphids, aleyrodids, cochineals, mites, lepidopteran and coleopteran larvae (Tavares *et al*. 2010).

Sustainable pest management covers compatibility of bio-agents with insecticides (Aliyu *et al.* 2017). EPNs and lady beetles as bioagents may be present in corn ecosystems that routinely receive chemical pesticides used for managing fall army worm. The selection of soft insecticide is in urgent need to conserve natural enemies. Novel insecticides having different mode of action are developed for management of fall army worm. However, the effect of these chemicals on EPNs and lady beetles are not studied. Information regarding safety of these natural enemies against used novel insecticides in corn ecosystem may open the scope for their integration for integrated pest management (IPM) of fall army worm in corn. Acknowledging this, the current work is done to find out precious information on the fate of some frequently applied insecticides in corn regarding mortality of EPN (*Steinernema* sp.) and lady beetle (*Cheilomenes sexmaculata*) under laboratory condition.

## MATERIALS AND METHODS

The research was conducted at *spring* season of 2021 under laboratory condition, College of Agriculture (BCKV), Burdwan Sadar, West Bengal, India.

The host insect rice grain moth (*Corcyra cephalonica*) was reared in laboratory for production of EPNs. NBAIIR (National Bureau of Agricultural Important Insect Resources), ICAR, Bangalore supplies the initial culture of *C. cephalonica* (National Accession No. NBAII-MP-PYR-01). Wheat flour was sterilized at $125^0$C for 1 hour in a hot air oven. After cooling at room temperature, 2.5 kg floor keeping in a wooden box was sprayed with 0.1% formalin and mixed with 40 g roasted groundnut powder and 5 g yeast. Each box is kept in rearing room ($30\pm2^0$C temperature and 70±5 % RH) after equal sprinkling of 0.25 CC fresh Corcyra egg over the food. Emerged adults were shifted to the egg laying chamber for oviposition.

The starting culture of *Steinernema* sp. (EPN) was collected from NIPHM (National Institute for Plant Health Management), Hyderabad, India. It was used for mass culturing on the last instar larvae of *Corcyra cephalonica* in the laboratory. Ten matured larva per petri plate lined with absorbent paper are inoculated with 2 ml water suspension having 500 infective juveniles (IJs) of *Steinernema* sp. The infected insects are transferred after 7 days to a moist filter paper on a concave side up watch glass surrounded with water in a petri plate (White 1927). The newly produced IJs migrate to water, where they are trapped and subsequently harvested for the study.

The grubs (late instar) and pupa of *Cheilonemes sexmaculata* were collected from insecticide untreated long bean fields of instructional farm of College of Agriculture, BCKV, Burdwan campus. These were directly used for this experiment. Treated grubs were provided with eggs of laboratory reared *Corcyra cephalonica* for their food.

Ten novel insecticides (Table 1) recommended for managing corn fall army worm (*S. frugiperda*) were evaluated in this study along with control using distilled water only. The stock solutions (500 ml of

**Table 1.** Detailed list of different insecticides.

| Treatment | Chemical name | Formulation dose (ml or g/500 lit of water) | Commercial name | Manufactured by | Mode of action* | Chemical group |
|---|---|---|---|---|---|---|
| $T_1$ | Cyantraniliprole 10.26 OD | 1350 | Benevia | FMC | RRM | Anthranilic Diamides |
| $T_2$ | Chlorantraniliprole 18.5 SC | 300 | Coragen | FMC | RRM | Anthranilic Diamides |
| $T_3$ | Emamectin benzoate 5SG | 330 | Proclaim | Syngenta | CCA | Avermectins |
| $T_4$ | Spinosad 45SC | 255 | Tracer | DOW | NARAA | Spinosyns |
| $T_5$ | Flubendiamide . 20WG | 225 | Takumi | TATA | RRM | Diamides |
| $T_6$ | Teflubenzuron 15 SC | 300 | Nomolt | BASF | CSI | Benzoyl phenylurea |
| $T_7$ | Novaluron 5.25 + indoxacarb 4.5 SC | 562.5 | Plethora | AADAMA | CSI+VSB | Benzoyl phenylurea+ Oxadiazine |
| $T_8$ | Broflanilide 30 SC | 93 | Exponus | BASF | GCAA | Meta-diamides |
| $T_9$ | Chlorfenapy 24 SC | 487.5 | Intrepid | BASF | DAP | Halogenated pyrroles |
| $T_{10}$ | Azadirachtin 1 EC | 3000 | Nimbecidine | T. Stanes | EA/M | Botanicals |
| $T_{11}$ | Water | - | - | - | - | - |

*Ryanodine receptor modulator (RRM), Chloride channel activators(CCA), Nicotinic acetylcholine receptor Allosteric activators (NARAA), Chitin synthesis inhibitor (CSI), Voltage-dependent sodium channel blockers (VSB), GABA-gated $Cl^-$ channel allosteric modulator (GCAA), Distrupt ATP production (DAP), Ecdysone agonists/moulting (EA/M).

each) of all these insecticides were prepared at 1.5 times higher concentration for each insecticide than recommended dose.

The below mentioned tactics was followed to assess EPNs's compatibility with *S. frugiperda* registered insecticides to search IJs' viability and infectivity. Entomo Pathogenic Nematodes (EPNs) were produced using late instar larva of *C. cephalonica* and then it was maintained in aqueous suspension keeping at room temperature.

To evaluate IJs' viability, 10 ml EPN solutions having 3000 IJs were taken in each Petridish with 3 replications for each treatment. Then respective insecticide at its target doses (1.5 times higher than recommended doses) is mixed with it using micropipette. In the control treatment, no insecticide is added. Survival of EPN was assessed after 48 hours of treatment. Now, 1ml of this suspension was taken and kept in nematode counting disk. This disk is divided into some equal sized small squares. One ml suspension of nematode-insecticide-water solution covered 10 numbers of such squares. The numbers of live nematodes was counted under microscope from five such small squares for each replication under each treatment.

To evaluate nematode infectivity, each petriplate layered with tissue paper received ten last instars larvae of *C. cephalonica* with 2 ml suspension of EPN (300 IJs/ml) and respective insecticides with 3 replications for each treatment. Then the plates were retained at laboratory with normal room temperature and humidity for seven days. Death larvae were counted and observed under microscope for presence of EPNs.

Bioassay on both stages (grub and pupa) of predatory coccinellid (*C. sexmaculata*) was done

following topical method of insecticide application. Thirty (30) randomly selected grubs for each replication were positioned separately in a covered petri plate (30 cm diameter) and sprayed directly with two mL of respective insecticide solution. Each set including untreated was repeated for thrice. These plates having predatory insects were dehydrated using room fan for few minutes before lid covering . The process of pupal bioassay was almost same like grub stage. But, here only twenty (20) numbers of coccinelid pupa were kept in petridish. Mortality response in case of grub was recorded up to three days at 24 hrs interval. Pupal mortality was calculated based on numbers of pupa not to transform into adult coccinelid.

Mortality data of EPNs and coccinelid was corrected using Abbott (1925) formula. Insecticides were grouped according to IOBC (International Organization for Biological Control) protocol of Hassan (1992). The following formula was used to calculate percent reduction in EPNs' infectivity to *Corcyra*.
RED = (1- It/Ic)×100, where RED = Percentage of infectivity reduction in the treatment

It = mortality in the treatments, Ic =mortality in control treatment

The experiment was conducted adopting Completely Randomized Design (CRD) and the data were analyzed by using the OPSTAT program.

## RESULTS AND DISCUSSION

Mortality of *Steinernema* sp. varied significantly for all the treatments than untreated control (Table 2). The highest mean corrected mortality revealed in $T_3$ (Emamectin benzoate – 43.03 %) followed by $T_4$ (Spinosad – 24.75 %) and $T_7$ (Novaluron+Indoxacarb – 13.05 %). It was lowest in $T_2$ (Chlorantraniliprole – 7.31 %) followed by statistically at par $T_5$ (Flubendiamide – 8.3 %), $T_1$ (Cyantraniliprole - 9.64 %), $T_8$ (Broflanilide - 9.83 %), $T_{10}$ (Azadirachtin – 10.52 %), $T_6$ (Teflubenzuron - 10.93 %) and $T_9$ (Chlorafenapyr - 11.55 %). Following IOBC protocol apropos EPN mortality, all the treatments belonged to harmless class except $T_3$ as slightly harmful one.

Infectivity i.e. the power of *Steinernema* sp. to cause larval death of *C. cephalonica* varied significantly after treated with different insecticides (Table 2). After incorporation of insecticides with the said EPN suspension, the highest (100 %) reduction in

**Table 2.** Effect of *Spodoptera frugiperda* registered insecticides on the mortality of Entomo-pathogenic Nematode (EPN) *Steinernema* sp. in laboratory conditions (Temperature 25±2ºC, Relative Humidity 70±5%).

| Treatment | Avg Cor Mortality (E) % | Toxicity category** | Infectivity % of *C. cephalonica* larva by insecticide treated *Steinernema* sp. | RED (Percentage of infectivity reduction) |
|---|---|---|---|---|
| $T_1$ | 9.64 (18.43) | 1 | 100.00 (90.00)* | 0.00 (4.05) |
| $T_2$ | 7.31 (16.03) | 1 | 100.00 (90.00) | 0.00 (4.05) |
| $T_3$ | 43.03 (41.26) | 2 | 0.00 (4.05) | 100.00 (90.00) |
| $T_4$ | 24.75 (29.59) | 1 | 76.67 (61.20) | 23.33 (28.77) |
| $T_5$ | 8.34 (17.19) | 1 | 100.00 (90.00) | 0.00 (4.05) |
| $T_6$ | 10.93 (19.74) | 1 | 80.00 (63.41) | 20.00 (26.55) |
| $T_7$ | 13.05 (21.57) | 1 | 66.67 (54.76) | 33.33 (35.20) |
| $T_8$ | 9.83 (18.71) | 1 | 93.33 (81.14) | 6.67 (11.55) |
| $T_9$ | 11.55 (20.26) | 1 | 20.00 (26.06) | 80.00 (63.90) |
| $T_{10}$ | 10.52 (19.37) | 1 | 96.67 (83.85) | 3.33 (8.85) |
| $T_{11}$ | 0.00 (4.05) | 1 | 100.00 (90.00) | 0.00 (4.05) |
| SE(m) ± | 1.94 | - | 3.61 | 3.11 |
| CD (p= 0.05) | 5.73 | - | 10.67 | 9.18 |

$T_1$: Cyantraniliprole 10.26 OD, $T_2$: Chlorantraniliprole 18.5 SC, $T_3$: Emamectin benzoate 5SG, $T_4$: Spinosad 45SC, $T_5$: Flubendiamide 20WG, $T_6$: Teflubenzuron 15 SC, $T_7$: Novaluron 5.25 + Indoxacarb 4.5 SC,$T_8$: Broflanilide 30 SC, $T_9$: Chlorfenapyr 24 SC, $T_{10}$: Azadirachtin 1 EC, T11: Water.

*The data within parenthesis is angular transformed value.

**Classes: 1 = Harmless (E < 30%), 2 = Slightly Harmful (30 < E < 79%), 3 = Moderately Harmful (80 < E < 99%), 4 = Harmful (E > 99%).

infectivity (RED) was documented in $T_3$ (Emamectin benzoate – 100 %) followed by $T_9$ (Chlorfenapyr – 80 %). The same was lowest (0 %) for four treatments such as $T_1$ (Cyantraniliprole), $T_2$ (Chlorantraniliprole), $T_5$ (Flubendiamide) and $T_{11}$ (Untreated control) followed by significantly at par $T_{10}$ (Azadirachtin – 3.33 %) and $T_8$ (Broflanide – 6.67 %). The other treatments like $T_6$ (Teflubenzuron), $T_4$ (Spinosad) and $T_7$ (Novaluron+Indoxacarb) resulted 20, 23.33 and 33.33 % RED, respectively.

Considering IOBC protocol and data recorded in Table 3, it bared that mean larval corrected mortality of *C. sexmaculata* was harmless (toxicity class-1) for most of the treatments such as $T_2$ (Chlorantraniliprole – 2.25 %). $T_4$ (Spinosad – 12.92 %), $T_5$ (Flubendiamide - 4.80 %), $T_6$ (Teflubenzuron - 17.58 %), $T_7$ (Novaluron + Indoxacarb – 28.6%), $T_8$ (Broflanide – 20.16 %), $T_9$ (Chlorfenapyr – 20.65 %) and $T_{10}$ (Azadirachtin – 13.11 %). The same was slightly harmful (Toxicity class 2) for rest two treatments like $T_3$ (Emamectin benzoate – 40.86 %) and $T_1$ (Cyantraniliprole – 39.65 %).

The substantial variation apropos corrected pupal mortality in different insecticidal treatments were accounted after comparing with untreated control (Table 3). The highest mortality was detected in $T_3$ (Emamectin benzoate – 30.74 %) followed by significantly at par $T_7$ (Novaluron + Indoxacarb – 30.37 %). It was lowest in $T_2$ (Chlorantraniliprole – 10.00 %) followed by significantly at par effect in $T_5$ (Flubendiamide – 13.33 %), $T_{10}$ (Azadirachtin – 16.67 %) and $T_4$ (Spinosad – 16.67 %). The other four treatments such as $T_1$ (Cyantraniliprole), $T_7$ (Chlorafenapyr), $T_8$ (Broflanilide) and $T_6$ (Teflubenzuron) caused respective

**Table 3.** Effect of *Spodoptera frugiperda* registered insecticides on the mortality of lady beetle in laboratory conditions (Temperature 25±2ºC, Relative Humidity 70±5%).

| Treatment | Cor. Mortality (%) of grubs | | | | Toxicity class** | Cor. Mortality (%) of pupa | Toxicity class |
|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | Mean | | | |
| 1 | 13.49 (21.95)* | 51.15 (45.93) | 54.32 (7.75) | 39.65 | 2 | 27.41 (31.18) | 1 |
| 2 | 2.22 (8.87) | 2.22 (8.87) | 2.30 (7.96) | 2.25 | 1 | 10.00 (10.45) | 1 |
| 3 | 13.49 (21.95) | 52.30 (46.59) | 56.79 (49.18) | 40.86 | 2 | 30.74 (33.51) | 2 |
| 4 | 4.48 (12.76) | 4.64 (12.96) | 29.63 (33.25) | 12.92 | 1 | 16.67 (22.99) | 1 |
| 5 | 2.22 (8.87) | 2.30 (7.96) | 9.88 (18.72) | 4.80 | 1 | 13.33 (20.86) | 1 |
| 6 | 3.33 (10.29) | 19.79 (26.74) | 29.63 (33.25) | 17.58 | 1 | 20.37 (26.80) | 1 |
| 7 | 10.08 (18.83) | 34.57 (36.29) | 40.72 (39.92) | 28.46 | 1 | 30.37 (33.47) | 2 |
| 8 | 3.37 (11.34) | 22.22 (28.41) | 34.89 (36.49) | 20.16 | 1 | 23.70 (29.02) | 1 |
| 9 | 7.85 (16.74) | 23.23 (29.10) | 30.86 (34.04) | 20.65 | 1 | 20.37 (26.65) | 1 |
| 10 | 2.22 (8.87) | 18.52 (25.77) | 18.60 (25.78) | 13.11 | 1 | 16.67 (24.25) | 1 |
| 11 | 0.00 (4.05) | 0.00 (4.05) | 0.00 (4.05) | 0.00 | - | 0.00 (4.05) | - |
| SE (m) ± | 1.78 | 1.76 | 1.30 | - | - | 2.52 | - |
| CD (p= 0.05) | 5.25 | 5.20 | 3.82 | - | - | 7.45 | - |

$T_1$: Cyantraniliprole 10.26 OD, $T_2$: Chlorantraniliprole 18.5 SC, $T_3$: Emamectin benzoate 5SG, $T_4$: Spinosad 45SC, $T_5$: Flubendiamide 20WG, $T_6$: Teflubenzuron 15 SC, $T_7$: Novaluron 5.25 + Indoxacarb 4.5 SC,$T_8$: Broflanilide 30 SC, $T_9$: Chlorfenapyr 24 SC, $T_{10}$: Azadirachtin 1 EC, $T_{11}$: Water.

*The data within parenthesis is angular transformed value.

**Classes: 1 = Harmless (E < 30%), 2 = Slightly Harmful (30 < E < 79%), 3 = Moderately Harmful (80 < E < 99%), 4 = Harmful (E > 99%).

mortality amounting 30.74, 30.37, 23.70 and 20.37 %. As per IOBC protocol, all of the treatments were harmless (Toxicity class 1) to pupa of *C. maculata* except $T_3$ and $T_7$ as slightly harmful (Toxicity class 2).

Cyantraniliprole resulted 55.6 % viability and 86 % infectivity of *S. feltiae* at 48 hrs after exposure (Guide *et al*. 2018). Koppenhoffer and Fugy (2008) suggested a highly safe IPM-compatible alternative using *H. bacteriophora*-chlorantraniliprole combinations for remedial white grub control. Harmless effect of chlorantraniliprole and flubendiamide on *Steinernema* sp. was also reported respectively by Patel 2021 and Devindrappa *et al.* 2017. Negrisoli *et al.* (2010) demonstrated mixing ability of Spinosad with entomopathogenic nematodes incuding *Steinernema.* In contrast, Eemamectin benzoate proved harmful to *S. carpocapsae* (Yan *et al.* 2012, Devindrappa *et al.* 2017). Concerning chitin synthesis inhibitor, diflubenzuron caused less effect for reproduction and development of *S. carpocapsae* (Hara and Kaya 1982, 1983). Similarly, here the same result was observed in another chitin synthesis inhibitor Teflubenzuron. All these earlier findings have direct support with the present results. Nematode's insensibility to the product may be resulting from the nematode's cuticle not having chitin in its structure, being constituted primarily by collagens, cuticulines, and other proteins.

Neem oil is recommended by Tavares *et al*. (2010) for control of *S. frugiperda* because of its high toxicity, combined with its relatively low toxicity (25.00 ± 0.33 %) to larvae of the coccinelid *E. connexa*. Spinosad had no lethal effects on larvae of two-spot ladybird, *Adalia bipunctata* (Jalali *et al.* 2009). Low (Spinosad) to moderate (Emamectin benzoate and indoxacarb) toxicity against coccinelid larva was confirmed Awasthi *et al.* (2013). Lady beetle predator (*C. septempunctata*) was more compatible with chlorantraniliprole than cyantraniliprole (Jiang *et al.* 2020). No report is available regarding effect of broflanilide against larval stages of any coccinelids. It is acknowledged for the first time by the present author. Earlier research may be insufficient on fate of lady beetle larva by rest of the insecticides such as chlorfenapyr, flubendiamide and teflubenzuron. Similarly, not so much effort earlier is taken to find out lady beetles' pupal lethality for most of the above mentioned insecticides. But, their adults were tolerant to chlorfenapyr and Flubendiamide (Mori and Gotoh 2009, Ozawa and Uchiyama 2016).

All these above written research findings by previous great contributors undoubtedly support directly or indirectly the result of both EPN and lady beetle as obtained and reported in this present manuscript.

From the study, it indicates that major insecticides recommended for managing corn fall army worm (*S. frugiperda*) are safe for pest's bioagents EPN (*Steinernema* sp.) and lady beetle (*Cheilomenes sexmaculata*) . Accordingly, the verdict may be drawn for compatible use of safer chemicals with EPNs and lady beetles. Such integration helps us to move towards sustainable pest management conserving natural enemies by diminishing the associated burning 3 R's problems (resistance, resurgence, and replacement) in plant protection.

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