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# Interaction effects of entomopathogenic nematodes and insecticides for the management of grubs of *Holotrichia longipennis* and *Brahmina coriacea*

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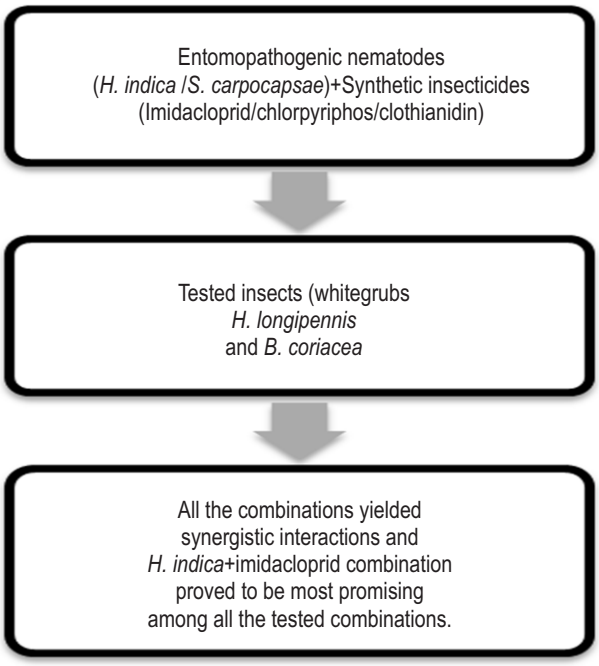
## Abstract

**Aim :** Laboratory studies were conducted to study the potential of entomopathogenic nematodes and their interaction with the commonly used insecticides *viz*., clothianidin, imidacloprid and chlorpyriphos against first, second and third instar grubs of *H. longipennis* and *B. coriacea.*

**Methodology :** Two entomopathogenic nematodes *viz*., *Heterorhabditis indica* Poinar and *Steinernema caprocapsae* Weiser were tested against I-III instar grubs of *Brahmina coriacea* (Hope) and *Holotrichia longipennis* (Blanchard) under laboratory conditions @1600IJs per cup by soil application. The mortality data was recorded after every 24 hrs up to 5 days. For evaluating the interaction effects among different tested biocontrol agents with insecticides, each entomopathogenic nematode species and insecticides was tested alone and in combination. *H. indica* and *S. carpocapsae* were applied @ 400IJs per cup. Insecticides were applied at a lower concentrations *viz*. 150, 100 and 75 ppm for chlorpyriphos, imidacloprid and clothianidin, respectively and were applied on the same day of nematode application with a gap of 2 hrs. Mortality data was taken after 24 hrs.

**Results :** Individual application of *H. indica* and *S. carpocapsae* @1600 IJs per cup had clearly lethal effect and produced mortality to the tune of 55.5-90.7 % in both the species of white grubs. *H. indica* revealed higher virulence as compared to *S. carpocapsae* against both the species. Combination of both the species with all the tested insecticides yielded synergistic interactions and *H. indica*+ imidacloprid combination proved to be the most promising among all the tested combinations.

**Interpretation:** Tested entomopathogens and insecticide combination produced synergistic interactions more effectively to first, second and early third instar grubs ( *H. longipennis* and *B. coriacea*).




**Key words:** *Brahmina coriacea,* Entomopathogens, *Holotrichia longipennis,* Synergistic interactions, White grubs

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## Introduction

White grubs are larvae of chafer beetles of the family Scarabidae which cause damage to a wide range of crops throughout the world. The world fauna of white grubs exceeds 30,000 species (Mittal, 2000), and the maximum number occurs in the tropical areas of the world, particularly in the African and Oriental regions. Larvae of greatest economic importance mainly belong to the tribe Melolonthini (Ritcher, 1961). It is a polyphagous pest, both in grub and adult stage and inflicts heavy damage on various fruit/forest trees, their nurseries, vegetables, lawns and field crops (Chandel and Kashyap, 1997; Mehta *et al.,* 2008). Grubs prefer to feed on fibrous roots for normal growth, and crops with tap root system suffer more as compared to those with adventitious root system (Yadav and Vijayvergia, 2000). Most species of white grubs complete their life cycle in one year and all scarabaeids without exception undergo three larval stages and spend more than half of their life time as larvae (Mehta *et al.,* 2010). In India, white grubs have now been included in the category of five national pests and are reported from every state causing damage to a wide variety of cultivated crops (Misra and Chandla, 1989). More than 1000 species of white grubs are known from the Indian sub-continent of which over 40 species attack a wide range of crop plants (Veeresh *et al.,* 1991). In Himachal Pradesh, about 20 species of white grubs have been reported to cause damage of 8-75 % in different crops (Chandel *et al.,* 2015; Pathania and Chandel, 2017).

Since this insect spends most of its life in the soil, except for a brief period of mating, the use of pesticide is often ineffective. Besides, excessive use of pesticides causes negative effects on the environment and human beings (Budak and Budak, 2006). According to Buss (2006) white grubs are among the most difficult soil insect-pests to control and highly resistant to insecticides. Thus, there is a need for the development of possible substitutes for the management of this pest. White grubs are naturally infected by various entomopathogens which kill their hosts or debilitate their future generations (Singh, 1991). Arora *et al.* (2000) reported that entomopathogens exert a controlling effect on grubs by means of their invasive properties, toxins, enzymes and other substances. Important entomopathogens which infect white grubs include fungi, bacteria, viruses and nematodes. Entomopathogenic nematodes have received more attention than other groups of microbial organisms with potential for use in the management of white grubs. Entomopathogenic nematodes, especially those which are obligate parasites of insects, possess desired attributes of a bio-control agent against white grubs.

Although many microbial agents have been evaluated against different white grub species, so far no serious attempt has been made to evaluate the effectiveness of entomogenous nematodes against the economically important white grub species of Himachal Pradesh. However, their implementation is constrained by different factors like, their availability is limited, they are relatively expensive, have limited persistence, and do not always provide consistent control levels (Georgis and Gaugler, 1991; Klein, 1993) and are also sensitive to many abiotic and biotic factors (Kaya, 1990; Kaya and Koppenhofer, 1996). Conceivably combining these entomopathogenic nematodes with recommended insecticides help us to understand the interaction of the components of an IPM program which will help to prevent failures in pest control, improve efficacy and also reduce the cost of the input (Bareth and Bhatnagar, 2013). The objective of this research was to study the synergistic/antagonistic effects of entomopathogenic nematodes in combination with different insecticides on white grubs. This study will pave a way to develop cost-effective management programme that incorporates pesticides and nonconventional management strategies for white grubs management under mid-hill conditions of Himachal Pradesh.

## Materials and Methods

Two species of entomopathogenic nematodes *viz., H. indica* and *S. carpocapsae* were tested against laboratory-reared I-III instars of two species of white grubs *viz., B. coriacea* and *H. longipennis.* Adults of *B. coriacea* were collected from walnut and apple trees in Shimla hills whereas adults of *H. longipennis* were collected from *toon* trees at Palampur. The collected beetles were transferred to mating glass jars (10.5x15.5 cm) filled with moist soil, and twigs of toon (*Toona ciliate* M. Roem.) and pear (*Pyrus communis* Linnaeus) were fixed in soil for feeding and mating of *B. coriacea* and *H. longipennis,* respectively. The beetles were daily fed with fresh leaves and the soil was examined for the presence of eggs. The eggs were separated with the help of moist camel's hair brush and were placed in Petri plate containing moist soil. In each Petri plate up to 25 eggs were placed and Petri plates were kept at room temperature.

**Rearing and maintenance of white grub culture in the laboratory:** 10-12 days old hatched eggs and freshly hatched grubs were transferred to small paper cups filled with moist soil containing 4-5 day old maize seedling whereas second and third instar grubs were fed on small potato tubers in the paper cups individually. First instar grubs were available from June to August and second instar grubs were available from last week of August to last week of September while third instar grubs were available during October and November. November onwards, the grubs started making earthen cells and slowed down their activities.

**Evaluation of entomopathogenic nematode agents against white grubs:** Two entomopathogenic nematodes *viz, H. indica* and *S. caprocapsae* were tested against grubs of *B. coriacea* and *H. longipennis* under laboratory conditions. *H. indica* was received in cadavers of *Galleria mellonella* (Linnaeus) from Foundation for Agricultural Resources Management and Environmental Remediation (FARMER), Ghaziabad. The cadavers were stored in the refrigerator at 5-7 ºC. The culture of *S. caprocapsae* was obtained from the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru and were multiplied in the laboratory using *Corcyra cephalonica* (Stainton). The concentration of nematodes per ml of suspension was

determined by counting the nematodes in counting dish under a stereo-zoom microscope. The average of four counts was taken to estimate the final nematode population per ml. The concentration of *H. indica* and *S. caprocapsae* was recorded as 195 IJs $ml^{-1}$ and 255 IJs $ml^{-1}$, respectively. The concentration of entomopathogenic nematodes was adjusted to 160 IJs $ml^{-1}$ and 10 ml of this suspension was taken in a dropper and was put into the soil directly in the cups containing grubs. This treatment was applied to a set of 15 grubs which was replicated three times. The insect mortalities were checked every 24 hrs, however, in tables mortality data of 1, 3 and 5 days of inoculation is given.

**Interaction effects of different entomopathogens in combination with different insecticides on white grubs:** For evaluating the interaction effects among different tested biocontrol agents with insecticides, each entomopathogenic nematode species and insecticides was tested alone and in combination. *H. indica* and *S. carpocapsae* were applied @ 400 IJs ml. Insecticides were applied at a lower concentrations *viz.,* 150, 100 and 75 ppm for chlorpyriphos, imidacloprid and clothianidin, respectively and were applied on the same day of nematode application at an interval of 2 hrs. Mortality data in each treatment was recorded after 24 hrs up to five days and mortality data was subjected to Abbott's formula for calculating corrected mortality. Synergistic, additive and antagonistic interactions between different treatments were determined by $\chi^2$ test (Rodriguez and Peck, 2009).

## Results and Discussion

Nematode species had a clear effect on mortality of both the species of white grubs when they were applied alone @1600 Ijs per cup. In case of *H. longipennis, H. indica* produced 66.7-90.7% mortality while *S. carpocapsae* produced mortality of 64.4-86.0% in first instar grubs after 1,3 and 5 days of treatment. Against second instar grubs, the mortality of 51.1-75.6 % and 55.6-73.3 % was caused by *H. indica* and *S. carpocapsae.* Against third instar grubs, the mortality data ranged from 44.4-66.7 % and 55.6-64.4 % in *H. indica* and *S. carpocapsae* treatment, respectively (Table 1). There was a gradual decrease in mortality over subsequent instars in both the species and the rate of mortality increased with the lapse of time. Against first instar grubs of *B. coriacea,* with *H. indica,* the mortality of 57.8-88.9 % was produced whereas with *S. carpocapsae,* 60.0-82.2 % mortality was recorded from 1-5 days of treatment. Treatment of second instar grubs with *H. indica* revealed 40.0-73.3 % mortality whereas *S. carpocapsae* treatment produced 44.4-68.9 % mortality after 1-5 days of treatment. In the case of third instar grubs, comparatively lower mortality was recorded and 33.3-64.4 %, and 31.2-55.5 % mortality was recorded with *H. indica* and *S. carpocapsae* treatments, respectively (Table 2).

The efficacy of *H. indica* decreased from first over second to third instar grubs. However, *S. carpocapsae* efficacy did not differ between second and third instars in *H. longipennis* but a decrease in susceptibility from younger to older instars was noticed in the case of *B. coriacea* grubs. The results of this study show that the efficacy of nematode species against different developmental stages of white grubs cannot be generalized and varies with species of nematode and white grubs. Similar observations were recorded by Koppenhofer and Fuzy (2004), who also observed that the efficacy of nematode species is not similar against all the species of white grubs. In another study conducted by Koppenhofer and Fuzy (2005), similar results were observed where *Heterorhabditis bacteriophora* Poinar and *Heterorhabditis zealandica* Poinar showed modest virulence to

**Table 1:** Mortality percentage of different instars of white grub, *H. longipennis* due to entomopathogenic nematodes *H. indica* and *S. carpocapsae*

| Instar | Observed mortality (%) after indicated days | | | | | |
|---|---|---|---|---|---|---|
| | *H. indica* | | | *S.carpocapsae* | | |
| | 1 | 3 | 5 | 1 | 3 | 5 |
| First | 66.7 | 80.0 | 90.7 | 64.4 | 75.6 | 86.0 |
| Second | 51.1 | 62.2 | 75.6 | 55.6 | 64.4 | 73.3 |
| Third | 44.4 | 55.6 | 66.7 | 55.6 | 64.4 | 64.4 |

**Table 2:** Mortality percentage of different instars of white grub *B. coriacea* due to entomopathogenic nematodes *H. indica* and *S. carpocapsae*

| Instar | Observed mortality (%) after indicated days | | | | | |
|---|---|---|---|---|---|---|
| | *H. indica* | | | *S.carpocapsae* | | |
| | 1 | 3 | 5 | 1 | 3 | 5 |
| First | 57.8 | 66.7 | 88.9 | 60.0 | 71.1 | 82.2 |
| Second | 40.0 | 48.2 | 73.3 | 44.4 | 55.6 | 68.9 |
| Third | 33.3 | 42.2 | 64.4 | 31.2 | 44.4 | 55.5 |

*Popillia japonica* Newman, *Anomala orientalis* Waterhouse, *Cyclocephala borealis* Arrow, and *Maladera castanea* (Arrow), and showed low virulence against *Rhizotrogus majalis* Razoumowsky. However, *Steinernema scarabaei* Stock and Koppenhofer showed lower virulence against *C. borealis,* but was highly virulent against *R. majalis, P. japonica, A. orientalis,* and *M. castanea.* In the case of both the species of entomopathogenic nematodes, the rate of mortality gradually increased with the lapse of time. This is in accordance with the studies of Kajuga *et al.* (2018) who tested the infectivity of local and exotic strains of *H. bacteriophora* and *S. carpocapsae* against white grubs. They observed that relative infectiousness of both the tested entomophogenic nematode species increased over the period of time.

Comparison among *H. indica* and *S. carpocapsae* against *H. longipennis* and *B. coriacea* revealed comparatively higher virulence of *H. indica* as compared to *S. carpocapsae* against all the instars. Marianelli *et al.* (2017) also observed that the strains of *H. bacteriophora* caused greater mortality (ranging from 57% to 100%) than those of *S. carpocapsae* (3% to 77%) against *P. japonica* grubs. Similarly, Singh and Gupta (2006) also reported that *H. bacteriophora* was more pathogenic than *Steinernema* species when tested against third instar grubs of *B. coriacea.* Bhatnagar *et al.* (2004) evaluated different entomopathogenic nematodes against grubs of *Maladera* insanabilis and they observed that *H. bacteriophora* was most pathogenic and lower inoculation doses of *H. bacteriophora* were required to kill the host. Contrary to our findings, Sharma *et al.* (2009) reported *S. carpocapsae* to cause higher mortality in the third instar grubs of *B. coriacea* as compared to *H. indica* after seven days of treatment.

Chi-square test showed synergistic interaction between entomopathogenic nematode species with all the tested insecticides when tested against both the species of white grubs. Entomopathogenic nematodes applied alone at lower concentration displayed some level of mortality. However, when entomopathogenic nematodes and insecticides were combined, the level of virulence was enhanced in a synergistic fashion. Synergistic levels of virulence were observed more in *H. indica* and insecticides combinations than in *S. carpocapsae* and insecticide combination. Additionally, synergy appeared to be more prevalent in the younger instars than the later instars and our findings are similar to the findings of Ansari *et al.* (2004) who indicated positive interactions with the combined application of entomopathogenic nematodes with the tested insecticides against *H. longipennis* and *B. coriacea grubs.*

In *H. longipennis,* individual application of insecticides, *i.e.,* clothianidin, imidacloprid and chlorpyriphos at lower concentrations produced 22.2-30.2, 17.8-32.6 and 20.0-34.1 per cent mortality in first, second and third instar grubs, respectively. *H. indica* and *S. carpocapsae* resulted in 22.2-34.9 and 20.0-30.2 per cent mortality when each entomopathogenic nematode was tested alone against I-III instar grubs of *H. longipennis.* The observed mortality in *H. indica*+clothianidin, *H. indica*+imidacloprid and *H. indica*+chlorpyriphos combination ranged from 57.8-75.0% mortality in I-III instar grubs of *H. longipennis* with expected mortality ranging from 39.47-54.56, 36.05-56.12 and 37.76-57.1%, respectively. The calculated $\chi^2$ values in all the tested combinations were higher than the tabulated value, hence synergistic type of interaction was recorded. In case of *S. carpocapsae* combination with insecticides, the observed mortality to the tune of 55.6-68.3, 53.3-72.8 and 51.1-70.5% was noticed in *S. carpocapsae* + clothianidin, *S. carpocapsae* +imidacloprid and *S. carpocapsae* +chlorpyriphos combination, respectively, and the expected mortality of 37.76-51.28, 34.24-52.95 and 36.0-54.0% was recorded in respective combinations. The value of $\chi^2$ was calculated to be more than the tabulated value, hence, indicating the synergistic interaction (Table 3).

Against the grubs of *B. coriacea,* clothianidin, imidacloprid and chlorpyriphos incurred 27.9-31.8, 22.2-24.4 and 15.6-20.0 % mortality in first, second and third instar grubs, respectively. *S. carpocapsae* produced 25.6, 24.4 and 26.7 % mortality against first, second and third instar grubs, respectively, when it was applied alone at a lower dose. The combination of both the entomopathogenic nematode species produced a synergistic effect against all the instars of both the species. However, the degree of synergism was comparatively less than those observed in *H. longipennis* grubs. *H. indica*+imidacloprid combination produced 64.4-84.1 and 44.46-60.21 observed and expected mortality in I-III instar grubs, and also produced the highest level of synergism among all the tested combinations. *H. indica*+ clothianidin and *H. indica* +chlorpyriphos produced observed mortality to the tune of 66.7-79.6% and 62.2-81.9%, respectively in I-III instar grubs. The $\chi^2$ value was recorded to be higher than the tabulated value, and interaction was found to be synergistic.

In the case of *S. carpocapsae* combined with imidacloprid, the observed mortality of 72.8, 60.0 and 64.4 % was recorded in first, second and third instar grubs. The expected mortality in *S. carpocapsae*+clothianidin combination mortality was recorded to be 54.87, 45.79 and 46.88 % against the first, second and third instar grubs, respectively. In *S. carpocapsae*+imidacloprid combination, the observed mortality of 77.3, 62.2 and 62.2% was recorded whereas expected mortality of 56.31, 44.22 and 43.96 % was noticed in the first, second and third instar grubs, respectively. *S. carpocapsae* +chlorpyriphos combination produced observed mortality of 75.1, 60.0 and 60.0 %, and expected mortality of 57.31, 45.79 and 45.42 % was seen in the first, second and third instar grubs, respectively. In all the instars, the combination of *S. carpocapsae* with all three tested insecticides synergistic interaction was recorded, as the calculated $\chi^2$ was higher than the tabulated value at one degree of freedom (Table 4).

The present study throws light on the synergistic interaction of imidacloprid, chlorpyriphos and clothianidin with *H.*

**Table 3:** Interaction of entomopathogenic nematode *H. indica* and *S. carpocapsae* with different insecticides against first, second and third instar grubs of *H. longipennis*

| Treatment | First instar | | | | Second instar | | | | Third instar | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $M_O$ | $M_E$ | χ2 | Interaction type | $M_O$ | $M_E$ | χ2 | Interaction type | $M_O$ | $M_E$ | χ2 | Interaction type |
| Clothianidin | 30.2 | - | - | - | 26.7 | - | - | - | 22.2 | - | - | - |
| Imidacloprid | 32.6 | - | - | - | 24.4 | - | - | - | 17.8 | - | - | - |
| Chlorpyriphos | 34.1 | - | - | - | 26.7 | - | - | - | 20 | - | - | - |
| *H. indica* | 34.9 | - | - | - | 26.7 | - | - | - | 22.2 | 0 | 0 | - |
| *H. indica*+Clothianidin | 75.0 | 54.56 | 7.66 | Synergistic | 64.4 | 46.27 | 7.14 | Synergistic | 57.8 | 39.47 | 8.49 | Synergistic |
| *H. indica*+Imidacloprid | 81.9 | 56.12 | 11.84 | Synergistic | 66.7 | 44.59 | 10.93 | Synergistic | 55.6 | 36.05 | 10.55 | Synergistic |
| *H. indica*+Chlorpyriphos | 77.3 | 57.1 | 7.15 | Synergistic | 64.4 | 46.27 | 7.1 | Synergistic | 53.3 | 37.76 | 6.42 | Synergistic |
| S. carpocapsae | 30.2 | - | - | - | 24.4 | - | - | - | 20 | - | - | - |
| *S. carpocapsae*+Clothianidin | 68.3 | 51.28 | 5.65 | Synergistic | 60.0 | 44.59 | 5.33 | Synergistic | 55.6 | 37.76 | 8.39 | Synergistic |
| *S. carpocapsae*+Imidacloprid | 72.8 | 52.95 | 7.44 | Synergistic | 62.2 | 42.85 | 8.76 | Synergistic | 53.3 | 34.24 | 10.65 | Synergistic |
| *S. carpocapsae*+Chlorpyriphos | 70.5 | 54 | 5.04 | Synergistic | 60.0 | 44.59 | 5.33 | Synergistic | 51.1 | 36 | 6.34 | Synergistic |

**Table 4:** Interaction of entomopathogenic nematode *H. indica* and *S. carpocapsae* with different insecticides against first, second and third instar grubs of *B. coriacea*

| Treatment | First instar | | | | Second instar | | | | Third instar | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $M_O$ | $M_E$ | χ2 | Interaction type | $M_O$ | $M_E$ | χ2 | Interaction type | $M_O$ | $M_E$ | χ2 | Interaction type |
| Clothianidin | 27.9 | - | - | - | 24.4 | - | - | - | 20 | - | - | - |
| Imidacloprid | 30.2 | - | - | - | 22.2 | - | - | - | 15.6 | - | - | - |
| Chlorpyriphos | 31.8 | - | - | - | 24.4 | - | - | - | 17.8 | - | - | - |
| *H. indica* | 27.9 | - | - | - | 22.2 | - | - | - | 31.1 | - | - | - |
| *H. indica*+Clothianidin | 79.6 | 58.9 | 7.27 | Synergistic | 68.9 | 51.31 | 6.02 | Synergistic | 66.7 | 47.36 | 7.9 | Synergistic |
| *H. indica*+Imidacloprid | 84.1 | 60.21 | 9.5 | Synergistic | 71.1 | 49.9 | 9.02 | Synergistic | 64.4 | 44.46 | 8.94 | Synergistic |
| *H. indica*+Chlorpyriphos | 81.9 | 61.13 | 7.03 | Synergistic | 68.9 | 51.31 | 6.02 | Synergistic | 62.2 | 45.91 | 5.78 | Synergistic |
| *S. carpocapsae* | 25.6 | - | - | - | 24.4 | - | - | - | 26.7 | - | - | - |
| *S. carpocapsae*+Clothianidin | 72.8 | 54.87 | 5.85 | Synergistic | 60.0 | 45.79 | 4.41 | Synergistic | 64.4 | 46.88 | 6.58 | Synergistic |
| *S. carpocapsae*+Imidacloprid | 77.3 | 56.31 | 7.84 | Synergistic | 62.2 | 44.22 | 7.33 | Synergistic | 62.2 | 43.96 | 7.59 | Synergistic |
| *S. carpocapsae*+Chlorpyriphos | 75.1 | 57.31 | 5.5 | Synergistic | 60.0 | 45.79 | 4.41 | Synergistic | 60.0 | 45.42 | 4.68 | Synergistic |

*indica* and *S. carpocapsae* in I-III instar grubs of *H. longipennis* and *B. coriacea.* Our results are in accordance with the studies of Polavarapu *et al.* (2007) who studied interaction between entomopathogenic nematode and neonicotinoid insecticides against grubs of *Anomala orientalis,* and recorded synergistic interaction of *H. bacteriophora* with sublethal doses of imidacloprid. Rodriguez and Peck (2009) also observed synergistic interaction of *H. bacteriophora* with neonicotinoids against third instar grubs of *Amphimallon majale.*

In this study, we were not able to determine which insecticide was synergized and which was synergist. Koppenhoffer *et al.* (2000) showed that the major factor responsible for synergistic interaction between imidacloprid and *H. indica* or *S. carpocapsae* is the sluggishness of imidacloprid treated grubs that facilitates host attachment and subsequent penetration. Due to disruptive behavioural effects, neonicotinoids are able to synergize the effect of other stressors. Neonicotinoids synergize the effect of other stressors in three ways *viz.,* first as antifeedant, second, they interfere with the insect nervous system to cause paralysis and third, they disrupt the normal nerve functions which interfere with defensive behaviours (Rodriguez and Peck, 2009; Koppenhoffer *et al.,* 2000). It can be concluded that the effect of combination of these EPNs with insecticides on white grubs must be investigated under field conditions. If the additive or synergistic effect of EPNs in combination with insecticides is confirmed under field conditions, they may offer a practical and safe method for the management of white grubs as an important tool in IPM programme.

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