

## Integrated use of entomopathogenic nematodes and selective insecticides for sustainable management of *Pieris brassicae* on cabbage

Atwa A. Atwa<sup>1\*</sup>, Marwa A. M. Abl-Allah, Ghada M. Ramadan and Heba S. Abd El-Aty

### ABSTRACT

The large cabbage white butterfly, or *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is a major pest of cruciferous vegetables, as it causes severe defoliation and economic loss in these vegetables because of heavy larval infestation. The study explored the pathogenicity of three entomopathogenic nematode (EPN) species, namely *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *Steinernema glaseri*, on the larval and pupal stages of *P. brassicae*; their compatibility against the selective insecticides Radiant (spinetoram) and Uphold (spinetoram/methoxyfenozide); and their effectiveness when used in mixtures against laboratory and field conditions. The bioassay experiments showed marked intraspecific variations among the nematode species in terms of virulence, with *H. bacteriophora* having the lowest value of LC<sub>50</sub> and LT<sub>50</sub> mortality on the two stages of *P. brassicae*, followed by *S. carpocapsae*, and relatively less virulence on *S. glaseri*. The compatibility experiments showed that the two selective insecticides have moderate to low toxicity against EPNs, and their mortality increased with concentration and exposure duration, but *H. bacteriophora* was more resistant than the *Steinernema* species. Mixes of nematodes given at the level of LC<sub>90</sub> and half of the recommended rate of insecticides showed additive to synergistic effects on larvae mortality of *P. brassicae* under laboratory bioassay conditions. The experiments conducted over two consecutive years (2024 and 2025) under the farm's actual agricultural conditions verified the findings and showed that the mix was more effective, achieving populations below 80%, and outperformed individual nematodes and insecticides. The findings show that the integrated use of *H. bacteriophora* entomopathogenic nematodes and smaller amounts of selective insecticides is a more effective and feasible method for controlling *P. brassicae* in cabbage production and justify their use within integrated pest management.

**Keywords:** *Pieris brassicae*; entomopathogenic nematodes; *Heterorhabditis bacteriophora*; Radiant; Uphold; compatibility; synergistic effects; integrated pest management.

**MS History:** 17.04.2026(Received)-22.05.2026(Revised)- 30.05.2026 (Accepted)

**Citation:** Atwa A. Atwa, Marwa A. M. Abl-Allah, Ghada M. Ramadan and Heba S. Abd El-Aty. 2026. Integrated use of entomopathogenic nematodes and selective insecticides for sustainable management of *Pieris brassicae* on Cabbage. *Journal of Biopesticides*, 19(1):21-31. DOI: 10.57182/jbiopestic.19.1.21-31

### INTRODUCTION

The great cabbage white, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is known to cause considerable damage to cruciferous vegetables, with heavy larval infestations resulting in severe foliar injury and economic yield loss. Chemical control remains the most prominent means of controlling *P. brassicae* populations in

agriculture (Kanwar *et al.*, 2025). However, such intensive use of chemical controls has been linked to several issues, including the development of resistance, detrimental effects on the population dynamics of natural enemies, and potential environmental pollution. Despite these constraints, there is a real need to diversify

environmentally acceptable methods for controlling *P. brassicae* within Integrated Pest Management (IPM) programs, alongside more efficient ones.

Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae have received considerable attention for their use in controlling insect pests in agricultural ecosystems. The EPNs actively search for their insect hosts and kill them through symbiotic association with certain bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.), which are released into the hemocoel after penetration. The bacteria multiply rapidly in the hemocoel, producing toxins and secondary metabolites that inhibit the host's immune system, causing septicemia and ultimately the host's death within 24 to 72 hrs after infection (Gaugler, 2002; Kaya *et al.*, 2006).

The use of EPNs is considered ideal for integration into IPM strategies because the organisms act quickly, pose no toxicity to vertebrates, have low residue issues, and have shown effectiveness against different lepidopterans under laboratory and field tests (Lacey and Georgis, 2012; Shapiro-Ilan *et al.*, 2017). EPN effectiveness is also dependent on species- and strain-specific traits, which include foraging behaviour, host-seeking activity, and infective rates. *Heterorhabditis bacteriophora* belongs to the group of cruiser foragers that actively search through soil or media to find hosts, while *Steinernema carpocapsae* demonstrates ambusher behaviour, involving high-speed host attachment via nictation, thereby often being more effective against hosts that are surface-active and foliage-associated (Lewis *et al.*, 2006). *Steinernema glaseri* typically shows lower infective rates under certain conditions but can be effective in certain host-environment combinations. Such intra- and interspecific variations in hosts' vulnerability and mortality rates continue to underscore the need to conduct comparative studies under specific conditions (Shapiro-Ilan *et al.*, 2014).

The coupling of EPNs with selective insecticides is a crucial element of IPM plans today to increase pest control efficacy and reduce insecticide use. Low-risk insecticides such as spinosyns and insect growth regulators (IGRs)

have gained popularity due to their selective modes of action and more acceptable toxin-risk characteristics (Sparks *et al.* 2012). Radiant® (spinetoram) and Uphold® (spinetoram and methoxyfenozide mix) have been increasingly used to combat lepidopteran pests, particularly *P. brassicae*, and are important for managing insecticide resistance. Previous studies have shown that some insecticides are compatible with EPNs, and additive or synergistic interactions can be achieved when insecticides and EPNs are applied simultaneously. Sublethal concentrations of insecticides may reduce host physiological resistance and immunity or alter host behavioural responses, thereby increasing the likelihood of nematode infection and penetration (Koppenhöfer and Fuzy, 2008; Negrisoni *et al.*, 2010). The interaction between EPN and insecticides is system-specific and depends on nematode species, insecticide formulation and concentration, exposure duration, and environmental conditions, which require empirical assessment under controlled laboratory and field conditions (Shapiro-Ilan *et al.*, 2014).

Although there is clear potential for integrating EPNs and insecticides, there is a lack of information on their integrated use against *P. brassicae* under Egyptian agroecological conditions. Within this context, this study aimed to: (i) test the pathogenicity of *H. bacteriophora*, *S. carpocapsae*, and *S. glaseri*, EPNs on larval and pupal *P. brassicae* within a controlled lab environment, (ii) test their compatibility with insecticides "Radiant" and "Uphold," (iii) investigate the impact of decreased concentrations of insecticide application, and (iv) test this approach's effectiveness within a natural setting. The sensitivity of this biological control method to environmental conditions and potential treatments could provide a scientific basis for implementing a biological control program for *P. brassicae* in cruciferous crops.

## MATERIALS AND METHODS

### *P. brassicae* collection

Last instar larvae and pupae of *P. brassicae* was collected from vegetable fields in Abu-Elnomros, Giza Governorate which is an inland governorate in northern Egypt, located on the

western bank of the Nile River and forming part of the Greater Cairo Region (approximately between 29° 45' and 30° 45' N latitude and 30° 45' and 31° 30' E longitude); to evaluate the virulence of the selected nematode isolates. Captured insect stages were directly placed individually in 30 mL plastic cups with screw plastic lids and transferred immediately to the laboratory.

### Nematode Strains

Three entomopathogenic nematodes, i.e. *Heterorhabditis bacteriophora* (Poinar) (Hp 88), *Steinemema glaseri* (Steiner, 1929) (NJ snaiu), and *Steinemema carpocapsae* (Weiser) (SC) were *in vivo* reared, in great numbers, on the last instar larvae of *G. mellonella* according to the technique of Dutky *et al.* (1964), and IJs were harvested from nematode traps as described by White (1927) at  $25 \pm 1$  °C. A stock suspension of IJs in sterilised distilled water at a concentration of 1000 IJs/mL was stored at 10 °C until needed. All nematodes were used within 14 days post-harvest, and a new stock was produced every 2 weeks.

### Insecticides

Two widely recommended insecticides from the Group 5 (Spinosyns – nAChR allosteric modulators) and Group 5 (Spinetoram) + Group 18 (Methoxyfenozide) were tested against the three EPN species, i.e. the Radiant (12% SC) provided by Corteva Agriscience, and Uphold (36% SC *H. bacteriophora*; *S. glaseri*; and *S. carpocapsae*) provided by Syngenta Agrochemical, Switzerland, according to their commercial formulation. Stock solutions and dilutions of these insecticides were freshly prepared in distilled water according to their formulations. Concentrations employed in this investigation were set at half, equal to, or 1.5 times the recommended field rate, which was 175 ppm for Radiant and 500 ppm for Uphold.

### Virulence Bioassays

The virulence of tested entomopathogenic nematode species, *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae*, was assessed against the late-instar larvae and pupae of *P. brassicae*. Comparative nematode infectivity was measured as LC<sub>50s</sub> (concentration of IJs required to kill 50% of treated insect stages) and LT<sub>50s</sub>

(time required to kill 50% of treated insect stages). The captured insect stages were washed with sterile distilled water and exposed to the nematode suspensions on the day of collection. The LC<sub>50s</sub> were determined by placing individual insect larvae/pupae in 30mL plastic cups with screw lids containing different concentrations of Dauer juveniles, ranging from 5 to 100, for each strain, on moistened filter paper (Whatman no. 1). Insects were covered with wet filter paper to provide sufficient moisture for nematode activity. Twenty *P. brassicae* larvae/pupae were tested under different nematode concentrations at  $25 \pm 2$  °C. Mortalities were recorded at 72 hrs post-infection, and cadavers were dissected to confirm nematode infections.

On the other hand, the LT<sub>50s</sub> were determined by exposing individual larvae and pupae to 100 Dauer juveniles of each nematode strain as described above. Twenty larvae and pupae of *P. brassicae* were examined for mortality at 12-hour intervals. The LC<sub>50s</sub> were then estimated on the log-concentration scale.

### Insecticides, nematodes, insect interactions

The investigation continued after identifying the most virulent nematode strains from the earlier bioassay experiments. Here, we assessed the toxicity of these pesticides to these strains and their capacity to kill hosts. Additionally, several toxicological studies were conducted to determine the insecticidal activity of this compound.

### Survival of insecticide-treated IJs

About 1000 IJs/mL of *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae* were used in this experiment. Three serial dilutions of each insecticide were prepared, viz., 87.5, 175, and 350 ppm for Radiant, and 250, 500, and 750 ppm for Uphold. Each insecticide dilution was added individually to Petri dishes (60 x 15mm) in 5-mL aliquots. Five mL of each nematode strain suspension was also added to obtain the desired mixture. Untreated controls were employed using distilled water in place of the insecticide solution. The Petri dishes were sealed with parafilm to avoid evaporation. Each treatment was replicated three times and incubated at  $25 \pm 2$  °C. Nematode survival was determined after

48 and 96 hrs of exposure to insecticides by taking a 1 mL sample from the mixed suspension in the Petri dishes. Nematodes that responded to prodding by movement were considered alive; those that did not move were recorded as dead (Hara and Kaya, 1983). Counts were repeated three times for each concentration at each time interval.

#### **Infectivity of insecticide-treated IJs**

The ability of the two insecticide-treated nematode strains to cause host mortality was tested at  $25 \pm 2$  °C against last-instar larvae of *P. brassicae*. The nematode inoculum for each strain was applied at a concentration equivalent to the  $LC_{90}$  for each larva to compare nematicidal potency. These  $LC_{90}$  values were derived from previous experiments on the log-concentration scale. A single larva of *P. brassicae* was placed on a 5.5 cm diameter filter paper in a 60 x 15mm petri dish containing 3 mL of nematode insecticide suspension, and exposed to combinations of  $LC_{90}$  for each IJs strain, plus 87.5 and 250 ppm (half the recommended dose) of Radiant and Uphold, respectively. Insect mortality was recorded 72 hours after initial exposure to the nematode insecticide suspension; all dead insects were dissected to verify that they harboured nematodes. Nematode concentrations that reached the  $LC_{90}$  and insecticide solutions were tested in parallel with the above treatments. Ten replicates were used per treatment.

#### **Field evaluation**

Field trials were designed based on the data extracted from the laboratory assays. Incompatible nematode-insecticide combinations were impractical for field work. Experiments were conducted at 6:00 PM, before sunset. Temperatures and relative humidity ranged from 14.8 to 32.4 °C and 28 to 91%, respectively. In all tests, water was used as the control solution in non-treated trees.

#### **Effect of Nematodes and/or Insecticides on *Pieris brassicae* Infestation**

In this experiment, a suspension of superfilm (1mL/litre) mixed with a suspension of *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae* (1000 IJs/mL) and/or a liquid formulation of Radiant and Uphold (175 ppm and 500 ppm in

single applications and 87.5 ppm and 250 ppm in nematode-insecticide combinations for both insecticides, respectively) was sprayed, using a 10-litre portable sprayer, at sunset to avoid excessive evaporation. These control agents were sprayed on cabbage (*Brassica oleracea* var. *capitata*) plants naturally infested with *P. brassicae* larvae under field conditions in the Abu-Elnomros region, Giza Governorate.

The experimental area ( $\frac{1}{2}$  feddan) was divided into many plots (25m<sup>2</sup> each). Each plot contained 100 cabbage plants. In November 2024 and 2025, twelve treatments were compared: a suspension of *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae* (150000 IJs/plant), aqueous liquid of Radiant and/or Uphold insecticide compounds (150000 IJs/plant + 10 ml insecticide/plant) and an untreated control (infested plants). In this test, each plot was considered as one replicate. Three replicates were used per treatment. Treated plots were arranged in a randomised complete-block design. Three days after application, the numbers of live and dead *P. brassicae* larvae per plot and per plant were determined. Dead larvae were collected and transported to the laboratory for dissection to assess nematode infectivity. Temperature and relative humidity were recorded during the experimental period.

#### **Data Analysis**

The data were normalised using an arcsine transformation. The significance of differences between the means was determined using analysis of variance (ANOVA). Comparisons were made by using Tukey's multiple range test. The  $LC_{50s}$  and  $LT_{50s}$  were estimated using the log-concentration scale. The percentage of insect mortality was calculated using the Henderson and Tilton formula (1955). Considering the joint action of each insecticide with each nematode species against *P. brassicae* infestation, the co-toxicity factor was calculated to determine whether the final effect of the combination was additive, synergistic, or antagonistic, using the equation of Sun and Johnson (1960).

Co-toxicity factor (C. F.) = Observed % mortality – Expected % mortality

**Expected mortality %**

A positive factor of (+20) or more means potentiation, a negative factor of (-20) or more means antagonism, and intermediate values (i.e., between -20 and +20) are considered additive effect.

**RESULTS AND DISCUSSION****Nematode Virulence against *P. brassica***

Laboratory bioassay data showed distinct variations in virulence among the tested species of the entomopathogenic nematode (EPN) against *P. brassicae* larvae and pupae. Glazer *et al.* (1997) explained that rapid mortality in insect hosts due to nematode infestation indicates high nematode virulence, attributed to a combination of faster penetration and rapid propagation of the symbiotic bacterium. In our assessment of the EPNs' virulence, we found that the LC<sub>50s</sub> and LT<sub>50s</sub> of all tested nematode species varied under laboratory conditions, depending on the insect stage and the EPN used. Among all the tested species, *H. bacteriophora* recorded the lowest LC<sub>50s</sub> (20 and 75 for both larvae and pupa, respectively) and LT<sub>50s</sub> (24 hrs and 36 hrs for both larvae and pupa, respectively), followed by *S. carpocapsae*, which took the same time to cause death of larvae and pupa but needed more concentration as the LC<sub>50s</sub> were 25 and 100, respectively. The lowest effectiveness and the longest time were recorded for *S. glaseri*. LC<sub>50s</sub> (50 and 150 for both larvae and pupae, respectively) and LT<sub>50s</sub> (36 hrs and 48 hrs for both larvae and pupae, respectively) were recorded. LC<sub>50</sub> data showed that *H. bacteriophora* had higher virulence than *S. carpocapsae*, followed by *S. glaseri*, which had the lowest virulence. Similarly, LT<sub>50</sub> values indicated that *H. bacteriophora* had higher infectivity and kill rates for larvae and pupae.

The increased efficacy of *H. bacteriophora* can be linked to its 'cruiser' foraging mode and its associated bacterium *Photorhabdus* spp., which multiplies rapidly in the hemocoel of the hosts, ultimately leading to septicemia and death (Gaugler, 2002; Kaya *et al.*, 2006). The other species, *S. carpocapsae* (an ambusher), although slightly slower in activity, and *S. glaseri*, requiring increased exposure times to cause the same levels of mortality as *H. bacteriophora*, agreed with previous studies pointing to the

increased effectiveness of *Heterorhabditis* spp. against lepidopteran larvae (Lacey and Georgis, 2012; Shapiro-Ilan *et al.*, 2017; Atwa and Hala, 2020). On the contrary, Atwa (2013) stated that *S. carpocapsae* has more potential against this pest than *H. bacteriophora*. Similar results were obtained by Elham *et al.* (2015), who reported that *S. carpocapsae* was more virulent than *H. bacteriophora* against *Z. pyrina* larvae under laboratory conditions. Considering the findings of those studies and our experiments, it could be suggested that the variation in the data might have resulted from the origins of the tested EPN species or from the techniques used.

The discrepancies among these results and those now presented can be ascribed to several interacting variables, namely strain origin, host species or developmental stage, bioassay design, and environmental parameters. In addition, the influence of feeding and symbiotic bacterial populations can differ among EPN species, thereby affecting infection efficiency and mortality rates. The cumulative evidence presented highlights that EPN species and system-specific efficacy are of prime importance in establishing which nematode species should be used in biocontrol programs.

**Insecticides Nematodes Pest Interactions****Survival of Insecticide-Treated IJs**

Nematode response to two insecticides (Radiant and Uphold) is presented in Table 1. As shown in this table, the three nematode species exhibited different survival capacities across insecticide concentration and exposure time. Evaluation indicated that nematode responses varied among insecticide groups and within groups. In contrast to conventional neurotoxic insecticides, Radiant (spinetoram) and Uphold (spinetoram + methoxyfenozide) demonstrated moderate to low toxicity to all tested nematode species. Nematode survival gradually declined with increasing concentration; however, survival percentages remained comparatively higher even at elevated concentrations. At 87.5 ppm, nematode survival generally exceeded 75%, while at 175 ppm, survival values ranged between approximately 65% and 46% for 350 ppm for *H. bacteriophora*, indicating a relatively lower toxic impact than that reported for Radiant

(spinetoram), while nematode survival was 68.0%, 59.0% and 42.0% for Uphold (spinetoram + methoxyfenozide) at 250 ppm, 500 ppm and 750 ppm, respectively, for *H. bacteriophora* (Table 1). However, the survival of EPNs against varying concentrations of Radiant and Uphold insecticides was significantly lower at higher concentrations and longer exposure times (Table 1). Even at a recommended concentration of 175

ppm for Radiant and 500 ppm for Uphold insecticides, nematode survival was significantly lower at 96 hours than at 48 hours for all nematode species tested. Among the nematode species tested, *H. bacteriophora* was more tolerant than *Steinernema carpocapsae* and *Steinernema glaseri* to both insecticides (Table 1).

**Table 1.** Effect of the two insecticide concentrations on nematode survival at two exposure periods

| Nematode species                     | Exposure period (hrs) | % of corrected nematode survival | % of corrected nematode survival | % of corrected nematode survival | % of corrected nematode survival | % of corrected nematode survival | % of corrected nematode survival |
|--------------------------------------|-----------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Nematode species                     | Exposure period (hrs) | Radiant                          | Radiant                          | Radiant                          | Uphold                           | Uphold                           | Uphold                           |
| Nematode species                     | Exposure period (hrs) | 87.5 ppm                         | 175 ppm*                         | 350 ppm                          | 250 ppm                          | 500 ppm*                         | 750 ppm                          |
| <i>Heterorhabditis bacteriophora</i> | 48                    | 75.0                             | 65.0                             | 46.0                             | 68.0                             | 59.0                             | 42.0                             |
| <i>Heterorhabditis bacteriophora</i> | 96                    | 63.0                             | 52.0                             | 34.0                             | 57.0                             | 42.0                             | 31.0                             |
| <i>Steinernema carpocapsae</i>       | 48                    | 69.0                             | 55.0                             | 41.0                             | 57.0                             | 43.0                             | 36.0                             |
| <i>Steinernema carpocapsae</i>       | 96                    | 48.0                             | 43.0                             | 31.0                             | 46.0                             | 34.0                             | 24.0                             |
| <i>Steinernema glaseri</i>           | 48                    | 68.0                             | 53.0                             | 39.0                             | 53.0                             | 41.0                             | 36.0                             |
| <i>Steinernema glaseri</i>           | 96                    | 49.0                             | 42.0                             | 30.0                             | 43.0                             | 32.0                             | 23.0                             |

\* Recommended field rate.

Radiant (spinetoram) was perceived as less toxic to EPNs than Uphold, as evidenced by higher survival at equal exposure times. This result is supported by previous findings that spinosyn insecticide formulations can be safely used when applied concurrently with EPNs, though at lower concentrations (Negrisoli *et al.*, 2010; Koppenhöfer and Fuzy, 2008). However, nematode survival was significantly affected by exposure time, underscoring the importance of timing for the effective use of optimised chemical and biological controls.

The lower intrinsic toxicity of Radiant and Uphold is due to their distinct modes of action, which are unrelated to acetylcholinesterase inhibition. Spinetoram is an allosteric modulator of the nicotinic acetylcholine receptor (IRAC

Group 5), inducing hyperexcitation only in insects, while methoxyfenozide is an ecdysone receptor agonist (IRAC Group 18), disrupting moulting processes in Lepidopteran larvae. Neither of these modes of action directly affects nematode neuromuscular systems, which may account for the relatively higher tolerance observed across all nematode strains. These results agree with earlier studies indicating that insecticides that show selectivity via a non-AChE-based mechanism are generally less harmful to entomopathogenic nematodes (Zimmerman and Cranshaw, 1990; Gordon *et al.*, 1996). Even with this grouping, differences in susceptibility among nematode strains were evident, which suggests that at least one component of tolerance to modern insecticides

involves genetic variability.

The effect of exposure duration on nematode survival was limited and depended on concentration, not duration. Extending the time beyond 48 hrs did not significantly affect survival rates. It appears that the two active ingredients, Radiant and Uphold, act for a limited period during which the nematodes exhibit a stable response towards the toxicity. This is similar to the findings of Saleh and Sammour (1995) and Gordon *et al.* (1996), which indicate that the effect of insecticides on nematodes occurs during the initial period and does not increase significantly thereafter.

Such tolerance could indicate the presence of a refractory subpopulation within some nematode isolates. As discussed by Gordon *et al.* (1996), susceptible individuals experience effects earlier, whereas tolerant individuals can tolerate exposure, potentially allowing the selection of a tolerant strain. Thus, it may be concluded that Radiant and Uphold are safe insecticides when used in combination with entomopathogenic nematodes, especially at field doses. Even at higher doses and longer exposure times (750 ppm for 96 hrs), nematode survival remained well within acceptable limits. Consequently, these insecticides can be considered effective in combination with entomopathogenic nematodes for controlling insect pests in IPM strategies. This agreed with Prakasa *et al.* (1975), Das and Divakar (1987), and Ishibashi and Takii (1993), which observed that nematodes used with insecticides at field doses showed no harmful interactions. Hara and Kaya (1983) also stated that mixtures of nematodes and insecticides may be effective in providing additive or supplementary mortality of insect pests and could be an effective approach for reducing dependence on chemical insecticides.

#### **Synergistic effects of joint EPN and insecticide treatments under lab conditions**

The simultaneous use of entomopathogenic nematodes (EPNs) at LC<sub>90</sub> doses combined with half doses recommended for use in the field for Radiant or Uphold significantly increased larval mortality of *Pieris brassicae* compared with each treatment used separately. In all cases involving a combination of EPNs and insecticides, significant larval mortality occurred within 48

hours, indicating additive and synergistic effects in laboratory experiments.

The likely cause for the increase in larval mortality is the cumulative effect of several factors. Sublethal dosing with insecticides such as spinetoram and methoxyfenozide has been shown to reduce insect physiological fitness by inhibiting general and specific enzyme systems, including cytochrome P450 monooxygenases, esterases, and glutathione S-transferases. This slows the insect's overall immune response, making it more susceptible to nematode infestation (Fan *et al.*, 2023). Consequently, growth inhibitors such as methoxyfenozide can disrupt the endocrine system and the moulting mechanism.

Another critical contributor to the development of synergism in the entomopathogenic nematode system involves the inhibition of the insect's immune mechanisms. Sublethal stress from the insecticide can cause decreased hemocyte concentration, reduced mechanisms of encapsulation, as well as disruptions in the phenol oxidase system, all of which are major players in the immune mechanisms within the insect against entomopathogenic nematodes and their bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) (Shapiro-Ilan *et al.*, 2017; Brivio and Mastore, 2018).

Behavioural changes induced by insecticide exposure could also increase nematode efficacy. Sublethal doses of spinosyn-type insecticides have been shown to reduce feeding activity, cause lethargy, and impair mobility in lepidopteran larvae, thereby increasing the likelihood of exposure to infective juveniles and reducing behavioural protection through activities such as grooming and avoidance (Laznik *et al.*, 2018). In addition, stress from insecticides can stimulate respiration and open spiracles, creating additional openings through which nematodes can enter.

Tangential or physical injury to the cuticle or midgut epithelium caused by insecticide use can also facilitate nematode entry. Several experiments have shown that these compounds can weaken the cuticle or disrupt the epithelial layer, facilitating EJ entry and increasing the rate of septicemia due to bacterial invasion of the

hemocoel following nematode entry (Koppenhöfer *et al.* 2020).

These synergistic effects observed in the current study are consistent with an increasing number of published reports showing that EPNs exhibit enhanced efficacy when used in mixtures with lower concentrations of selective insecticides against lepidopteran larvae. Similar effects have also been found in *Spodoptera litura*, *Helicoverpa armigera*, *Plutella xylostella*, and *Spodoptera frugiperda*, wherein mixtures caused an increase in mortality as well as the reduction of LT<sub>50</sub> and the dose of nematodes needed (Shapiro-Ilan *et al.*, 2014; Lacey *et al.*, 2015; Koppenhöfer *et al.*, 2018).

In integrated pest management (IPM) terms, such synergy can be highly beneficial. The use of reduced insecticide doses in combination with EPNs can lower chemical inputs, delay the development of resistance, preserve non-target organisms, and enhance environmental safety while maintaining or improving pest control efficacy (Shapiro-Ilan *et al.*, 2017; Vermelho *et al.*, 2024). These findings support the strategic inclusion of EPN–insecticide combinations as a sustainable component of IPM programs targeting *P. brassicae* and other economically important lepidopteran pests.

#### **Field efficacy of EPNs and insecticide combinations against *P. brassicae***

This was confirmed by trials involving entomopathogenic nematodes (EPNs) conducted over the 2024 to 2025 growing seasons, which showed that they could effectively control *Pieris brassicae* under natural conditions (Table 2). The percentage reductions of 63.2 to 74.8% were recorded when applied singly and outperformed those of other species when carried out by *Heterorhabditis bacteriophora*. This can partly be attributed to their foraging pattern and subsequent penetration levels of *H. bacteriophora* compared to other species, together with the quick-killing action of their bacterium, *Photorhabdus* (Campos-Herrera *et al.*, 2016; Shapiro-Ilan *et al.*, 2017).

However, when used singly at lower dosages, both Radiant and Uphold showed only moderate effectiveness, with population reductions of no more than 51.8%. This can be attributed to the fact that lower insecticide dosages, although

environmentally friendly, have not achieved an effective reduction of Lepidopteran pests when used alone (Vermelho *et al.*, 2024; Sparks *et al.*, 2021).

It is pertinent to note that the combined use of EPNs at half the insecticide rate consistently achieved the highest pest control, with levels above 80% in most instances across both seasons. The negative co-toxicity factor (CF) values are significant indicators of synergistic effects; for example, *H. bacteriophora* paired with Radiant or Uphold. From these results, it is apparent that by combining both conventional and novel control tools, high levels of efficacy can be realized even in open fields with varying environmental regimes.

The sustainability of these synergistic effects over two consecutive years attests to the reliability and stability of this holistic strategy. The seasonal nature of these effects could indicate that EPN–insecticide mixtures are not affected by temperature, soil moisture, or sun exposure, despite these factors influencing EPN survival and efficacy in the environment (Campos-Herrera *et al.*, 2015; Koppenhöfer *et al.*, 2018). The EPN's capacity to recycle within target populations and inhabit these soil environments could be an additional factor.

Mechanistically, the increased field efficacy of these combined treatment approaches can be attributed to partially non-overlapping and time-staggered modes of action. Insecticides exert rapid killing and subsequent effects. Larval mobility and larval feeding are suppressed by insecticides soon after treatment, thus lowering damage to crops. Surviving larvae, which had undergone sublethal dosages of insecticides, are far more susceptible to infection by nematodes, and this effect is supplemented by EPNs through infection, reproduction, and secondary cycling in the target pest population (Shapiro-Ilan *et al.*, 2014; Koppenhöfer *et al.*, 2020).

In the context of IPM strategies, the use of EPNs alongside reduced insecticide levels offers several highly valuable advantages. Firstly, it decreases the total use of chemicals, hence minimizing the risks associated with non-target species, pollinators, and natural enemies, which are typically found in cruciferous systems.

**Table 2.** Effect of different EPN species and/or Radiant or Uphold on *P. brassica* infesting cabbage plants (*Brassica oleracea* var. capitata) in November 2024 and 2025.

| Treatments<br>(nematode and/<br>or insecticides)<br>+ superfilm | November 2024                                    |                                                   |                                     |       | November 2025                                    |                                                   |                                     |       |
|-----------------------------------------------------------------|--------------------------------------------------|---------------------------------------------------|-------------------------------------|-------|--------------------------------------------------|---------------------------------------------------|-------------------------------------|-------|
|                                                                 | No. of<br>larvae/40<br>plants pre-<br>treatments | No. of<br>larvae/40<br>plants post-<br>treatments | % of<br>populatio<br>n<br>reduction | C.F.* | No. of<br>larvae/40<br>plants pre-<br>treatments | No. of<br>larvae/40<br>plants post-<br>treatments | % of<br>populatio<br>n<br>reduction | C.F.  |
| <i>H. bacteriophora</i>                                         | 222                                              | 61                                                | 72.5                                | -     | 234                                              | 59                                                | 74.8                                | -     |
| <i>S. carpocapsae</i>                                           | 225                                              | 79                                                | 64.9                                | -     | 246                                              | 78                                                | 68.3                                | -     |
| <i>S. glaseri</i>                                               | 242                                              | 89                                                | 63.2                                | -     | 227                                              | 76                                                | 66.5                                | -     |
| Radiant 87.5<br>ppm                                             | 243                                              | 126                                               | 48.2                                | -     | 276                                              | 133                                               | 51.8                                | -     |
| Uphold 250<br>ppm                                               | 235                                              | 143                                               | 39.2                                | -     | 285                                              | 149                                               | 47.7                                | -     |
| Radiant 175<br>ppm                                              | 234                                              | 63                                                | 73.1                                | -     | 279                                              | 68                                                | 75.6                                | -     |
| Uphold500 ppm                                                   | 231                                              | 77                                                | 66.7                                | -     | 281                                              | 82                                                | 70.8                                | -     |
| <i>H. bacteriophora</i> +<br>Radiant<br>87.5ppm                 | 267                                              | 41                                                | 81.4                                | -18.4 | 290                                              | 38                                                | 84.2                                | -31.7 |
| <i>S. carpocapsae</i> +<br>Radiant<br>87.5ppm                   | 231                                              | 39                                                | 79.4                                | -19.4 | 248                                              | 37                                                | 83.0                                | -32.1 |
| <i>S. glaseri</i> +<br>Radiant<br>87.5ppm                       | 239                                              | 36                                                | 82.1                                | -18.2 | 250                                              | 39                                                | 82.4                                | -33.8 |
| <i>H. bacteriophora</i> +<br>Uphold 250ppm                      | 231                                              | 38                                                | 81.3                                | -20.4 | 243                                              | 38                                                | 81.2                                | -27.0 |
| <i>S. carpocapsae</i> +<br>Uphold<br>250ppm                     | 241                                              | 68                                                | 68.1                                | -32.5 | 284                                              | 78                                                | 69.1                                | -37.3 |
| <i>S. glaseri</i> +<br>Uphold250ppm                             | 231                                              | 39                                                | 79.4                                | -22.8 | 283                                              | 38                                                | 84.1                                | -25.2 |

\* Co-toxicity factor (C. F.) = Observed % mortality – Expected % mortality

Secondly, resistance development to the used insecticides remains a major worldwide concern for cruciferous insects, including both *P. brassicae* and *P. xylostella*, and selecting EPNs and reduced levels of other chemicals minimizes this problem (Campos-Herrera *et al.*, 2016; Lacey *et al.*, 2015). Thirdly, the use of EPNs and reduced levels of other chemicals aligns with the requirements of sustainable agriculture goals and regulatory trends favouring reduced-risk pest management tools.

Similar synergistic field performance of EPN–insecticide combinations has been reported in cabbage and other cruciferous crops, where these strategies achieved effective suppression of lepidopteran pests while conserving beneficial arthropods and improving yield outcomes (Lacey *et al.*, 2015). Collectively, these findings

support the inclusion of EPN–insecticide combinations as a practical, environmentally sound component of IPM programs for *P. brassicae* management under field conditions.

## REFERENCES

- Atwa A. Atwa, 2013. Susceptibility of *Spodoptera littoralis* (Boisd.) to treated entomopathogenic rhabditids, *Heterorhabditis bacteriophora* and *Steinernema* sp. by different pesticides. *Journal of Biopesticides*, **6**(2):149-159.
- Atwa, A. A., and Hala, A. 2020. Compatibility of three virulent Egyptian entomopathogenic nematode strains and two organophosphorus insecticides in controlling *Zeuzera pyrina* L. in olive orchards. *Amazonian Journal of Plant Research*. **4** (2):559-571.

- Brivio, M. F., and Mastore, M. 2018. Nematobacterial complexes and insect hosts: Different Weapons for the Same War. *Insects*, **9**(3): 117. <https://doi.org/10.3390/insects9030117>
- Campos-Herrera, R., El-Borai, F. E., Martín, J. A. R. and Duncan, L. W. 2016. Entomopathogenic nematode food web assemblages in Florida natural areas. *Soil Biology and Biochemistry*, **93**: 105-114.
- Campos-Herrera, R., El-Borai, F. E. and Duncan, L. W. 2015. Modifying soil to enhance biological control of belowground dwelling insects in citrus groves under organic agriculture in Florida. *Biological Control*, **84**: 53-63.
- Das, J. N. and Divakar, B. J. 1987. Compatibility of certain pesticides with DD-136 nematode. *Plant Protection Bulletin*. **39**: 20-22.
- Dutky, S. R., Thompson, J. V. and Cantwell, G. E. 1964. A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology*, **6**: 417-422.
- Elham, S., Javad, K., Hussein S. N. and Mojtaba H. 2015. Efficacy of two entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* for control of the leopard moth borer *Zeuzera pyrina* (Lepidoptera: Cossidae) larvae under laboratory conditions. *Biocontrol Science and Technology*, **25**(3): 260 – 275.
- Gaugler, R. (Ed.), 2002. Entomopathogenic Nematology. CABI Publishing, Wallingford, UK, 388 **PP**.
- Glazer, I., Salame, L. And Segal, D. 1997. Genetic enhancement of nematocide resistance in entomopathogenic nematodes. *Biological Science and Technology*. **7**: 499-512.
- Gordon, R., Chippett, J. and Tillay, J. 1996. Effects of two carbomates on infective juveniles of *Steinernema carpocapsae* all strain and *Steinernema feltiae* umea strain. *Journal of Nematology*, **28**(3): 310-317.
- Hara, A. H. and Kaya, H. K. 1983. Toxicity of selected organophosphate and carbamate pesticides to juveniles of the entomogenous nematode *Neoaplectana carpocapsae* (Rhabditida: Steinernematidae). *Environmental Entomology*, **12**: 496-501.
- Ishibashi, N. And Takii. S. 1993. Effects of insecticides on movement, nictation, and infectivity of *Steinernema carpocapsae*. *J. of Nematology*, **25**(2): 204-213.
- Kaya, H. K., Aguilera, M. M., Alumai, A., Choo, H. Y., de la Torre, M., Foder, A., Ganguly, S., Hazir, S., Lakatos, T., Pye, A., Wilson, M., Yamanaka, S., Yang, H., and Ehlers, R. U., 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biol. Control*, **38**: 134–155.
- Kanwar, P. S., Chander, P. and Rasna, G. 2025. Pest of brassica's: challenges and sustainable solutions. *Plant Archives*, **25**(2): 1512-1518. DOI:10.51470/PLANTARCHIVES.2025.v25.supplement-2.190.
- Koppenhöfer, A. M., Fuzy, E. M., 2008. Effect of the anthranilic diamide insecticide, chlorantraniliprole, on *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) efficacy against white grubs (Coleoptera: Scarabaeidae). *Biol. Control*. **45**: 93–102.
- Koppenhöfer, A. M., Kostromytska, O. S., and Wu, S. H. 2018. Pyrethroid-resistance level affects performance of larvicides and adulticides from different insecticide classes in population of *Listronotus maculicollis* (Coleoptera: Curculionidae). *J. Econ. Entomol.* **111**: 1851–1859. DOI: 10.1093/jee/toy142.
- Koppenhöfer, A. M., Shapiro-Ilan, D. I., and Hiltbold, I. 2020. Advances in the use of entomopathogenic nematode biopesticides in suppressing crop insect pests, in *Biopesticides for Sustainable Agriculture*, (eds.) Birch, N. and Glare, T. (Cambridge: Burleigh Dodds Science Publishing), 1–38. doi: 10.19103/AS.2020.0073.10
- Lacey, L. A., and Georgis, R. 2012. Entomopathogenic nematodes for control of insect pests above and below ground. *Journal of Nematology*, **44**: 218–225.
- Vermelho, A. B., Moreira J. V., Akamine I. T., Cardoso V. S., and Mansoldo F. R. P. 2024.

- Agricultural pest management: the role of microorganisms in biopesticides and soil bioremediation. *Plants (Basel)*, **13**(19): 2762. doi: 10.3390/plants13192762. PMID: 39409632; PMCID: PMC11479090.
- Lacey L. A., Grzywacz D., Shapiro-Ilan D. I., Frutos R., Brownbridge M. and Goettel M. S. 2015. Insect pathogens as biological control agents: Back to the future. *J. Invert. Pathol.* **132**: 1–41. doi:10.1016/j.jip.2015.07.009
- Laznik, Z., Bohinc, T., and Trdan, S., 2018. Applicability of invasive alien plants in controlling harmful organisms of cultivated plants. *Acta Agric Slovenica*. **111**: 501-509. <https://doi.org/10.14720/aas.2018.111.2.22>.
- Lewis E. E., Campbell, J., Griffin, C., Kaya, H. and Peters, A. 2006. Behavioral ecology of entomopathogenic nematodes. *Biological Control*, **38**(1): 66 -79.
- Fan, R. F., Sun, Z., Chen, Y., and Gui, F. 2023. Insecticide Susceptibility and Detoxification Enzyme Activity of *Frankliniella occidentalis* under Three Habitat Conditions. *Insects*, **14**(7): 643. <https://doi.org/10.3390/insects14070643>
- Negrisoni, J. A. S., Garcia, M. S., Barbosa, N. C., Bernardi, D., and Silva, A., 2010. Efficacy of entomopathogenic nematodes (Nematoda: Rhabditida) and insecticide mixtures to control *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) in corn crops. *Crop Prot.* **29**: 677–683.
- Prakasa Rao, P. S., Das, P. K. and Padhi, G. 1975. Note on compatibility of DD-136 (*Neoplectanadutkyi*), an insect parasitic nematode, with some insecticides and fertilizers. *Indian Journal of Agricultural Sciences*. **45**: 275-277.
- Saleh, M. M. E. and Sammour, E. A. 1995. Interactions of three insecticides and two entomopathogenic nematodes against *Spodoptera littoralis* (Boisd.) larvae. *Egyptian Journal of Biological Pest Control*, **5**(2): 119-122.
- Shapiro-Ilan, D. I., Lewis, E. E., and Schliekelman, P. 2014. Aggregative group behavior in insect parasitic nematode dispersal. *Int. J. Parasitol.* **44**: 49–54. doi: 10.1016/j.ijpara.2013.10.002
- Shapiro-Ilan, D., Arthurs, S. P., and Lacey, L. A. 2017. Microbial control of arthropod pests of orchards in temperate climates, in *Microbial Control of Insect and Mite Pests*, (ed.) Lacey, L. A. (Amsterdam: Elsevier), 253–267. doi: 10.1016/B978-0-12-803527-6.00017-2
- Sparks, T. C., Dripps, J. E., Watson, G. B., and Paroonagian, D. 2012. Resistance and cross-resistance to the spinosyns – a review and analysis., *Pestic. Biochem. Physiol.* **102**: 1–10.
- Sparks, T. C., Storer, N., Porter, A., Slater, R. and Nauen, R. 2021. Insecticide resistance management and industry: the origins and evolution of the Insecticide Resistance Action Committee (IRAC) and the mode of action classification scheme. *Pest Manag. Sci.* **77**: 2609–2619
- Steiner, G. 1929. *Neoplectana glaseri* n. g., n. sp. (Oxyuridae). A new nematode parasite of the Japanese beetle. *J. Wash. Acad. Sci.*, **19**: 436-440.
- Sun, W. P. and Jhonson, E. R. 1960. Synergistic and antagonistic actions of pesticides-synergistic companion and their mode of actions. *J. Agric. Food chem.* **8**(4): 261-266.
- Zimmerman, R. J. And Cranshaw, W. C. 1990. Compatibility of three entomogenous nematodes (Rhabditida) in aqueous solutions of pesticides used in turfgrass maintenance. *J. of Economic Entomol.* **83**(1): 97-100.

---

**Atwa A. Atwa\*, Marwa A. M. Abl-Allah, Ghada M. Ramadan and Heba S. Abd El-Aty**  
 Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza 12619, Egypt

**\*Corresponding Author:**

E mail- atwaradwan@arc.sci.eg

Tel: +20100 510 9816 & + 202 3570 4359