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Compatibility of entomopathogenic nematodes and chemical insecticides mixture on controlling diamondback moth, *Plutella xylostella*

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Abstract
One of the challenges in integrating entomopathogenic nematodes (EPNs) with chemical control is that farmers frequently abuse insecticides due to limited reliable information. The purpose of this study was to determine the impact of combining the recommended doses (RD) of insecticides on the efficacy of EPNs against diamondback moth, *Plutella xylostella* larvae as well as looking into differences in the combined action of EPN species and chemical insecticides. The bioefficacy test of the EPN- insecticide mix revealed that indoxacarb had a potentiation response and was less toxic to infective juveniles of *Heterorhabditis indica* (RS5 and RTR); on the other hand, an additive impact was observed with H. *indica* (SR2, GA6, and TG1). Deltamethrin and dichlorvos showed severe toxicity effects to infective juveniles in all combinations after being mixed with the H. *indica* isolates. The tested chemical insecticides and Infective Juveniles of EPNs, showed an additive and potentiating effect and there was no indication of an antagonistic reaction with all combinations. Overall, results indicate the feasibility of the integrated use of these nematode species and chemical pesticides in crop protection.

Keywords: Entomopathogenic nematodes, diamondback moth, *Plutella xylostella*, *Heterorhabditis indica*, infective juveniles

1. Introduction
The diamondback moth (DBM), *Plutella xylostella* (L.), is the most destructive pest of cruciferous vegetable crops in the world, including in India (Kianpour *et al.*, 2014) [14]. The larvae in their first instar mine in to the leaf, eat and skeletonize it, which hinders the plant's ability for growth and makes it unfit for consumption. According to Krishnamoorthy (2004) [16], the diamondback moth caused a 52% yield reduction in cabbage in India. Its infestation causes a 30-100% loss in cole crops in India (Ahmed *et al.*, 2009) [2]. The annual cost of managing the diamondback moth is anticipated to be US$ 4-5 billion (Zalucki *et al.*, 2012) [32]. Indiscriminate use of synthetic insecticides for the management of this pest has led to the development of insecticide resistance (Talekar *et al.*, 1990) [30]. Among the various bio-agents, entomopathogenic nematodes were found to be relatively effective than others in suppressing the population of diamondback moth.
In addition, entomopathogenic nematodes can effectively control a number of agriculturally important lepidopteran, coleopteran, and dipteran pests (Shapiro-Ilan and Gaugler, 2002) [28]. EPNs utilize bacteria carried in their alimentary canal to kill their insect hosts (Poinar, 2018). EPNs are sprayed to kill insect pests that feed on above -ground parts (Arthurs *et al.*, 2004) [5]. A more economical and efficient management option can be obtained by combining many control agents, which can also increase the effectiveness of IPM techniques. When two control agents work separately on a target host and the toxicity of one component is unaffected by the other, their combined effects can either be additive, potentiating, or antagonistic (Robertson *et al.*, 2017) [26]. Research indicates that combining biological control agents with low-impact insecticides or lower insecticide concentrations may increase the effectiveness of the biocontrol agents while reducing the adverse effects of the insecticides (El-Ashry and Ramadan 2021) [7]. On the other hand, it has also been suggested that exposure to certain chemicals may promote nematode motility, improve host-finding behaviour, and increase host penetration (Ishibashi and Takii, 1993) [13]. According to Laznik *et al.*, (2012) [18], tank mixing of EPNs and insecticides may have additive or, preferably, synergistic effects on pest mortality.

The knowledge about the compatibility between chemical pesticides and EPNs can play an important role in developing and improving the foliar application. Therefore, the compatibility of different agrochemicals and EPN isolates had been assessed to achieve efficient pest management.

2. Methods and Materials

2.1 Rearing of DBM

The initial culture of the DBM, was established by collecting large numbers of larvae from cultivated field of cabbage and cauliflower from Horticultural Research Instructional Farm, IGKV Raipur. The larvae were reared on fresh cabbage leaves in an insect breeding dish in the BOD incubator at 25 ±2°C and 70-75 per cent relative humidity. Pupae were separated and transferred to another rearing cage covered with muslin cloth for adult emergence, adults were given a 10% honey solution soaked in an absorbent cotton swab as diet and cabbage leaves were provided for oviposition. Egg-bearing leaves were removed and placed in another plastic container for hatching. Fresh cabbage leaves were fed to the instars until they pupated. The culture of *P. xylostella* was multiplied and maintained during the experimental period, according to protocol mentioned by Harika *et al.*, (2019) [12].

2.2 Insecticides used for bioassay

To determine the compatibility of EPNs, nine commercial insecticides registered for the management of DBM in cabbage *viz*., Emamectin benzoate 5% SG (0.3 g/l), Imidacloprid 17.8% SL (0.5 ml/l), Fenvalerate 20.6% EC (2.5 ml/l), Deltamethrin 2.8% EC (1 ml/l), Diclorvos 76% EC (0.5 ml/l), Indoxacarb 15.8% EC (0.5 ml/l), Flubendiamide 39.35% SC (1 ml/l), Chlorantraniliprole 18.5% SC (0.4 ml/l), Cypermethrin 25.8% EC (0.5 ml/l) were evaluated.

2.3 Compatibility bioassay studies between EPNs and insecticides against *Plutella xylostella*

2.3.1 Preparation of control

A stock solution of infective juveniles and distilled water was prepared with a concentration of 200 Infective Juveniles (IJs)/ml.

2.3.2 Compatibility test of EPNs with different insecticides

The insecticide solutions were prepared as per the recommended doses. One ml of insecticide solution and one ml of nematode suspension were poured into each three wells of the 6-well culture plate. The dishes were sealed with parafilm, arranged in a completely randomized design (CRD), and incubated at room temperature. The treatments were replicated three times. The mortality of IJs was observed after 48 h by taking 10µl aliquots of nematode suspension from each well and observing them under the stereo zoom microscope. Nematodes that did not move even after prodding were considered dead, and the motile or 'S' shape of IJs was considered a live IJs.

$$\text{Survivability percent} = \frac{\text{Number of live IJs}}{\text{Total Number of IJs}} \times 100$$

2.3.3 Virulence test of EPNs with different insecticides on DBM

For the virulence assay, last-instar larvae of P. *xylostella* were used as test insects. The virulence test was performed only for insecticide-treated IJs. After 48 hrs exposure to insecticides, IJs were rinsed four times with distilled water to remove insecticidal resiudes. The LC_{25} concentration (Sunanda *et al*., 2014) [29] of nematodes for DBM was prepared, followed by the LC_{50} of each insecticide solution. Each insecticide solution (LC_{50}) was mixed with the stock solution (LC_{25}) of IJs. 1 ml of prepared solution containing IJs and insecticide was poured into petri dishes lined with filter paper, and ten larvae of *P. xylostella* were released in each petri dish provided with cabbage leaves. The treatments were replicated three times. These petri dishes were kept at room temperature, and larval mortality was observed 48 hours after application. The dead insects were dissected in Ringer's solution to confirm their death by EPN. After that, the co-toxicity factor was evaluated for comparing insecticides.

$$\text{Mortality Percent} = \frac{\text{Number of dead larvae}}{\text{Total Number of larvae}} \times 100$$

$$\text{Corrected Mortality Percent} = \frac{\text{T(Observed Mortality\%)} - \text{C(Control Mortality\%)}}{100 - \text{C(Control Mortality\%)}} \times 100$$

Where,
T=% mortality in treated larvae of DBM
C=% mortality in untreated larvae of DBM

The expected effect was compared with the observed effect to evaluate if the effects were additive, synergistic, or antagonistic, using the equation suggested by Mansour *et al.*, (1966) [19].

$$\text{Co-toxicity Factor} = \frac{\text{Observed mortality \% – Expected Mortality\%}}{\textit{Expected mortality\%}} \times 100$$

In this equation, observed mortality is the toxicity observed experimentally in combinations of IJs and insecticides. Expected mortality was the additive sum of the observed mortality of IJs and insecticides alone. This criterion was used to classify the results into three categories. A positive factor of 20 or more indicates potentiation; a negative factor of 20 or more indicates antagonism; and intermediate values between -20 and +20 indicate an additive effect (El Sobki *et al.*, 2020) [6].

2.4 Statistical analysis

A complete randomized design was implemented in all experiments. Statistical analysis was performed using SPSS 29.0. And OPSTAT. Insect mortality was corrected according to the control treatment values using Abbott's formula (Abbott, 1925). Prior to analysis, percentage data were arc-sine transformed. All experimental mean differences were considered significant at $p<0.05$. The median lethal concentrate (LC_{50}) values were calculated by probit analysis (Finney, 1971) [10] using Analyst soft Biostat Pro V 5.8.4.3 Software.

3. Result and Discussion

3.1. Effect of insecticide on EPNs survival under laboratory conditions

Five isolates of *H. indica* (RS5, RTR, TG1, GA6, and SR2) were subjected to an experiment to record the impact of several insecticides at recommended dose (LC_{50}) on entomopathogenic nematodes in controlled environment. The observation on the percentage of *H. indica* IJs that were still alive after 48 hours are given in Table: 1. The results showed that the insecticides with the highest mean survivability was Indoxacarb 15.8% EC (97.20%) followed by Emamectin benzoate 5% SG (95.28%), Chlorantraniliprole 18.5% SC (92.68%), Flubendiamide 39.35% SC (91.38%), Fenvalerate

20% EC (90.64%), Cypermethrin 25% EC (88.58%), Imidacloprid 17.8% SL (78.04%), Deltamethrin 2.8% EC (39.06%) and Dichlorvos 76% EC (0.82%). Since the treatment averages were statistically not equivalent to one another, there was discernible difference between them.

The present findings are in consonance with those of Yan *et al.,* (2012) who observed that the survivability of *H. indica* IJs were not affected by emamectin benzoate, but their infectivity was impaired. According to Fetoh *et al.,* (2009) [9] Emamectin benzoate had no negative effects on *H. indica*, and a mixture of H. *indica* with formulated emamectin benzoate significantly improved mortality to greasy cutworm (*Agrotis ipsilon*) compared to H. *indica* alone. As a result, prior to field applications in IPM programs, studies of the interaction and compatibility of insecticides and EPNs are recommended. Koppenhofer and Fuzzy, (2004) [15] reported that imidacloprid alone cause (45%) low mortality but when treated synergistically with *Steinernema scarabaei* (isolated from grub) caused 90 to 94 per cent white grub mortality in laboratory and field experiments. The current investigations are in accordance with the Negrisoli *et al.,* (2010) [20], who showed 88% survival of *H. indica* when treated to Cypermethrin. Negrisoli *et al.,* (2008) [21] found that when exposed to Deltamethrin, a pyrethroid, mortality of *H. indica* (28.4%) was higher than H. *bacteriophora* (5.6%). In parallel to the results recorded in this study by Amizadeh, (2019) [3], the dichlorvos treatment had the highest mortality rate on both isolates of S. *feltiae* (100%), followed by abamectin, indoxacarb, and chlorantraniliprole treatments, respectively. Many organophosphorus insecticides have been shown to exhibit nematicidal activity against various species of *Steinernema* and *Heterorhabditis* (Negrisoli *et al.,* 2010) [20]. It should be noted that different pesticides from the same chemical group may have varied effects on nematodes. As a result, the effect of synthetic compounds on EPNs cannot be determined only on the basis of their chemical group (Rovesti and Deseo, 1990) [27]. Furthermore, even when the EPNs and the two insecticides, chlorantraniliprole and indoxacarb, were simultaneously used, there was no evidence of antagonism.

The findings agree with results of Gordon *et al.,* (1996) [11], who observed that pesticides including organophosphates and carbamates were harmful to *Steinernema* spp. and *Heterorhabditis* spp., though with notable differences between the various active ingredients. Kumar *et al.,* (2015) [17] observed that highest mortality of IJ's was observed in Dichlorvos (72.0%). In the present study, the movement of infective juveniles was severely hampered within 24 hours of exposure to Dichlorvos, caused complete mortality and not included in infectivity assessments. Dichlorvos was found harmful against IJs as it inflicted high mortality rate, thus rendering them as ineffective component of IPM. Therefore, it was concluded that combinations of insecticide with EPN's were more effective than individual application. Due to the action of insecticides the insect larvae became sluggish and the larvae were more susceptible to nematodes attack.

Table 1: Survivability percentage of IJ's after 48 hr period to the recommended doses of chemical insecticides under laboratory conditions

Insecticides	Nematode Species (H. *indica*)					Mean
	RS5	RTR	TG1	GA6	SR2	
Emamectin benzoate 5% SG	96.59	94.52	94.87	94.86	95.55	95.28
	(79.36)*	(76.47)	(76.91)	(76.90)	(77.82)	
Imidacloprid 17.8% SL	79.53	77.39	76.79	78.43	78.08	78.04
	(63.10)	(61.61)	(61.20)	(62.33)	(62.08)	
Fenvalerate 20% EC	92.16	90.76	90.10	90.08	90.08	90.64
	(73.74)	(72.31)	(71.66)	(71.64)	(71.64)	
Deltamethrin 2.8% EC	40.62	38.37	37.90	39.37	39.06	39.06
	(39.59)	(38.28)	(37.99)	(38.86)	(38.68)	
Dichlorvos 76% EC	3.42	0.68	0.00	0.00	0.00	0.82
	(10.66)	(4.73)	(0.00)	(0.00)	(0.00)	
Indoxacarb 15.8% EC	97.96	97.95	96.59	96.23	97.27	97.20
	(81.79)	(81.76)	(79.36)	(78.81)	(80.49)	
Flubendiamide 39.35% SC	92.49	91.78	91.12	89.38	92.12	91.38
	(74.10)	(73.34)	(72.67)	(70.98)	(73.69)	
Chlorantraniliprole 18.5% SC	93.52	92.47	91.81	93.84	91.78	92.68
	(75.25)	(74.07)	(73.37)	(75.63)	(73.34)	
Cypermethrin 25% EC	89.43	88.70	88.05	87.68	89.04	88.58
	(71.03)	(70.36)	(69.77)	(69.45)	(70.67)	
Control (Distilled water)	100.00	100.00	100.00	100.00	100.00	100.00
	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	
Mean	78.57	77.26	76.72	76.99	77.30	
C.D.	4.90	3.81	3.75	3.53	5.68	
SE(m)	1.65	1.28	1.26	1.19	1.91	
*Figures in parentheses are Arc sine transformed values.						

3.2. Interactions between chemical insecticides and EPNs

Interaction results between chemical insecticides and different strains of EPNs in controlling the last instar larvae of diamondback moth are demonstrated in (Table 2).

Indoxacarb showed potentiation interaction with *H. indica (*RS5 and RTR) strains recorded Co-toxicity factors of +23.95 and +20 respectively. Whereas, indoxacarb mixed with H. *indica* (SR2, GA6 and TG1) showed additive interaction recorded +19.51, +19.01 and +18.52 as co-toxicity factors, respectively. Emamectin benzoate showed an additive effect after mixing with isolates RS5, RTR, SR2, TG1 and GA6, recorded a Co-toxicity factor of +15.06, +14.57, +14.07, +13.58 and +10.12, respectively. When *H. indica* isolates *i.e*., RS5, RTR, SR2, GA6 and TG1 were mixed with Chlorantraniliprole and Imidacloprid exhibited an additive effect with co-toxicity factor of +14.57, +11.11, +10.12,

+9.14, and +8.64 and co-toxicity factors of +6.67, +5.68, +5.19, +4.20, and +3.70, respectively. When the *H. indica* isolates RS5, RTR, SR2, TG1 and GA6 were mixed with flubendiamide and Cypermethrin showed an additive impact with co-toxicity factors of +5.68, +5.19, +4.69, +3.70, and +0.74 and co-toxicity factors of +6.17, +5.68, +5.19, +4.69, and +3.70, respectively. Fenvalerate recorded additive interaction after mixed with H. *indica* isolates RS5, RTR, GA6, SR2 and TG1 with co-toxicity factors of -3.70, -4.20, -4.69, -5.19 and -6.17 respectively. Deltamethrin exhibited high toxicity against the *H. indica* isolates RS5, RTR, GA6, SR2 and TG1, with co-toxicity factors of -8.15, -8.64, -9.14, -9.63 and -11.11 respectively.

Present findings were with the consonance of Anuar and Daniel, (2009) [4] who observed the synergistic interaction between H. *bacteriophora* and Imidacloprid against white grubs. The investigation results of this study was in conformity with the results of El Sobki *et al*., (2020) [6] who reported that against ninth instar larvae of *R. ferrugineus*, LC_{25} and LC_{50} of imidacloprid mixed with various strains of the tested EPNs showed potentiation interaction recorded Co-toxicity factor +36.80 and +23.73, respectively, while LC_{25} and LC_{50} of zuta cypermethrin mixtures showed additive interaction, with values of +16.40 and +3.46, respectively. Emamectin benzoate's LC_{25} and LC_{50} recorded an additive impact of +13.20 and +1.60, respectively.

Table 2: Interactions between chemical insecticides and different entomopathogenic nematode strains on mortality of the last instar larvae of Diamondback moth under laboratory conditions

Nematode species	Chemical Insecticides (LC_{50})	Mortality% (Nematode + insecticides)		Co-toxicity factor	Response
		Expected	Observed		
RS5 (LC_{25})	Emamectin benzoate 5% SG	75	86.30	15.06	Additive
	Imidacloprid 17.8% SL	75	80.00	6.67	Additive
	Fenvalerate 20% EC	75	72.22	-3.70	Additive
	Deltamethrin 2.8% EC	75	68.89	-8.15	Additive
	Indoxacarb 15.8% EC	75	92.96	23.95	Potentiation
	Flubendiamide 39.35% SC	75	79.26	5.68	Additive
	Chlorantraniliprole 18.5% SC	75	85.93	14.57	Additive
	Cypermethrin 25% EC	75	79.63	6.17	Additive
RTR (LC_{25})	Emamectin benzoate 5% SG	75	85.93	14.57	Additive
	Imidacloprid 17.8% SL	75	79.26	5.68	Additive
	Fenvalerate 20% EC	75	71.85	-4.20	Additive
	Deltamethrin 2.8% EC	75	68.52	-8.64	Additive
	Indoxacarb 15.8% EC	75	90.00	20.00	Potentiation
	Flubendiamide 39.35% SC	75	78.89	5.19	Additive
	Chlorantraniliprole 18.5% SC	75	83.33	11.11	Additive
	Cypermethrin 25% EC	75	79.26	5.68	Additive
TG1 (LC_{25})	Emamectin benzoate 5% SG	75	85.19	13.58	Additive
	Imidacloprid 17.8% SL	75	77.78	3.70	Additive
	Fenvalerate 20% EC	75	70.37	-6.17	Additive
	Deltamethrin 2.8% EC	75	66.67	-11.11	Additive
	Indoxacarb 15.8% EC	75	88.89	18.52	Additive
	Flubendiamide 39.35% SC	75	77.78	3.70	Additive
	Chlorantraniliprole 18.5% SC	75	81.48	8.64	Additive
	Cypermethrin 25% EC	75	77.78	3.70	Additive
GA6 (LC_{25})	Emamectin benzoate 5% SG	75	82.59	10.12	Additive
	Imidacloprid 17.8% SL	75	78.15	4.20	Additive
	Fenvalerate 20% EC	75	71.48	-4.69	Additive
	Deltamethrin 2.8% EC	75	68.15	-9.14	Additive
	Indoxacarb 15.8% EC	75	89.26	19.01	Additive
	Flubendiamide 39.35% SC	75	75.56	0.74	Additive
	Chlorantraniliprole 18.5% SC	75	81.85	9.14	Additive
	Cypermethrin 25% EC	75	78.52	4.69	Additive
SR2 (LC_{25})	Emamectin benzoate 5% SG	75	85.56	14.07	Additive
	Imidacloprid 17.8% SL	75	78.89	5.19	Additive
	Fenvalerate 20% EC	75	71.11	-5.19	Additive
	Deltamethrin 2.8% EC	75	67.78	-9.63	Additive
	Indoxacarb 15.8% EC	75	89.63	19.51	Additive
	Flubendiamide 39.35% SC	75	78.52	4.69	Additive
	Chlorantraniliprole 18.5% SC	75	82.59	10.12	Additive
	Cypermethrin 25% EC	75	78.89	5.19	Additive

Similar findings were reported by El-Ashry *et al*., (2020) [8] who reported that the high sensitivity of H. *bacteriophora* (HP88 strain) to certain chemicals showed up after exposure for one day to abamectin (14.2%) and fenamiphos (13.2%), followed by formulations of chlorpyrifos (12.2 and 11.2%), there was no significant mortality between the tested formulations. Finally, after two days of exposure, flubendiamide recorded 10.8% mortality with different significance with other treatments, except for chlorpyrifos (Tafaban 48% EC). Despite this, flubendiamide was considered the least toxic active ingredient on EPNs. After mixing with H. *bacteriophora* (Ba-1) and S. *feltiae* recorded (CF= -32.22 and -38.86), the antagonistic effect was seen. When combining flubendiamide with *H. bacteriophora*

(HP88), S. *glaseri*, and S. *carpocapsae*, showed additive interaction, with CF values of -12.58, -18.10, and -18.13, respectively. High toxicity and an antagonistic action have been reported by fenamiphos against S. *carpocapsae*, *S. glaseri*, H. *bacteriophora* (HP88), S. *feltiae*, and H. *bacteriophora* (Ba-1), with corresponding CF values of -26.83, -29.73, -38.39, -40.42, and -42.88, respectively.

4. Conclusion

Indoxacarb exhibited potentiation with all *Heterorhabditis indica* isolates. Positive additive effects between isolates and emamectin benzoate, chlorantraniliprole, flubendiamide, imidacloprid, and cypermethrin were noticed. However, fenvalerate and deltamethrin exhibited detrimental additive effects with isolates. As a precaution, mixing can be done as needed, although it is recommended to employ EPN after applying pesticides to prevent negative effects and maintain sustainability. The current research showed that *H. indica* can be effectively included into IPM. It might lessen reliance on chemical insecticides, which would help to delay the emergence of insecticide resistance and escape harmful consequences for the environment and public health. The findings of this study expand our knowledge of how well EPN works with registered insecticides to manage insect pests.

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