



***In vitro* Compatibility and Efficacy Studies of Entomopathogenic Fungi *Metarhizium anisopliae* (Metsh.) with Commonly used Biorational and Chemical Pesticides against *Spodoptera litura* (Fabricius)**

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i2430805

Editor(s):

(1) Prof. Al-kazafy Hassan Sabry, National Research Centre, Egypt.

Reviewers:

(1) Isela Quintero Zapata, Universidad Autonoma De Nuevo Leon, Mexico.

(2) Dr. Anakha Kaladharan, Vijaya Group of Hospitals, India.

Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here:
<https://www.sdiarticle5.com/review-history/78677>

Original Research Article

Received 12 October 2021
Accepted 15 December 2021
Published 24 December 2021

ABSTRACT

In vitro compatibility of selected entomopathogenic fungi with botanicals and chemical insecticides at field recommended concentrations Indoxacarb 14.5 SC, Spinosad 45 SC, neem oil and NSKE were non-toxic to the test strain *M. anisopliae* (Ma-L-1) as they did not show significant reduction in radial growth. The insecticide dichlorvos 76 EC (DDVP) recorded 100 per cent reduction in radial growth of test strains at field recommended concentration. The joint action of microbial agents (bacteria, viruses and fungi) revealed that the combination of pathogens did not prove superior to individual effect. All the combination of entomopathogenic fungi *M. anisopliae* (Ma-L-1) strain with microbial agents were within the critical limits of additive effect and combination with insecticides viz., Spinosad 45 SC @0.009%, neem oil 5% and NSKE 5%, which produced the synergism reaction.

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Keywords: *Metarhizium anisopliae*; indoxacarb 14.5 SC; spinosad 45 SC; monocrotophos 36 SL; chlorpyrifos 20 EC, dichlorvos 76 EC, *Spodoptera litura*, neem oil and NSKE.

1. INTRODUCTION

Insects like other organisms are susceptible to variety of diseases caused by viruses, bacteria, fungi, protozoans, rickettsia, phytoplasma and nematodes. Among them entomopathogenic fungi are gaining importance in the IPM schedules in recent years, although *Bacillus thuringiensis* (Bt.) and nuclear polyhedrosis viruses (NPV) are the most widely used microbials at present. More than 750 spp. of fungi from about 100 genera are pathogenic on insects, many of them offer great potential for pest management [1]. The fungal diseases on insects are commonly referred as "mycosis". Fungi infect insects of almost all orders, most common in Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera. The fungi can be used effectively in IPM under humidity (RH>70%) and moderate temperature (20 -30°C) on economically important crops.

Worldwide, there is a search for locally adapted strains of entomopathogenic (bacteria, viruses, fungi) for effective management of insect pests for that particular environment. In many cases successful control of insects have been achieved by using local strains rather than exotic microorganisms (strains). So, it is assumed that local strains of microorganisms might have well adapted to that particular environment from where they are isolated and may play major role. Species of *Spodoptera* are known to be attacked by almost all groups of entomopathogens. However, considerable work has been done only with NPV, which effectively control *S. litura* on crops such as tobacco (*Nicotiana tabacum*), cotton (*Gossypium hirsutum*), cabbage (*Brassica oleracea*), banana (*Musa acuminata*) and black gram (*Vigna mungo*) [2]. Epizootic of disease by entomopathogenic fungi *Beauveria*, *Metarhizium* and *Nomuraea* spp. have been reported as on *S. litura* in India [3,1]. Though in nature mixed infection of two or more entomopathogens is common, researchers have so far concentrated only on testing their individual efficacy. The control achieved by using entomopathogens individually is quite satisfactory in some cases and in some others it is not so encouraging, since these pathogens are greatly influenced by the environmental factors. Although pathogen have been widely tested individually. However, very few such studies have been conducted to evaluate their efficacy in combination.

Conceivably, such a combination could be advantageous when more than one species of lepidopterous pest occur on the same crop simultaneously. There is also a possibility that two pathogens might interact synergistically, one pathogen may predispose the pest species for higher degree of infectivity by another pathogen or they may act as supplementary and complimentary to one another or they may be antagonistic to one another [4]. The utility of microorganisms and other bio-control agents are over emphasized in Bio-intensive Integrated Pest Management (IPM). More over the compatibility between microorganisms and chemical pesticides are also criteria for using them in IPM. Keeping this in view the present study was carried out with the objective to study the compatibility of the entomopathogenic fungi with the available biorationals and chemical insecticides in laboratory.

2. MATERIALS AND METHODS

2.1 *In vitro* Compatibility of Selected Entomopathogenic Fungi with Botanicals and Chemical Insecticides against *Spodoptera litura*

2.1.1 Compatibility of selected entomopathogenic fungal isolate of *M. anisopliae* (Ma-L-1) with selected insecticides

The commonly used insecticides were tested *in vitro* for their inhibitory effect, if any on selected entomopathogenic fungal isolate of *M. anisopliae* in terms of radial growth following Poison Food Technique. The insecticides and their concentrations tested in this experiment are Indoxacarb 14.5 SC -0.0045 per cent, Spinosad 45 SC -0.018 per cent, Monocrotophos 36 SL - 0.310 per cent, Chlorpyrifos 20 -0.050 percent, Dichlorvos 76 EC (DDVP)-0.120 percent, Endosulfan 35 EC -0.350 per cent, Neemoil-5 per cent. Each test insecticide at field recommended concentration was tested with five replications.

2.1.2 Incorporation of test insecticides into the media

Sterilized SDAY (Sabouraud dextrose agar + yeast extract) medium was melted and cooled but before solidification, the test insecticides at field recommended concentration, Indoxacarb

14.5 SC -0.0045 per cent, Spinosad 45 SC - 0.018 per cent, Monocrotophos 36 SL -0.310 per cent, Chlorpyrifos 20 EC -0.050 per cent, Diclorovos 76 EC (DDVP)-0.120 per cent, Endosulfan 36 EC -0.350 percent, Neemoil-5 percent were added treatment wise by using micropipette. The medium was shaken vigorously for even mixing of the contents and poured into sterile petriplates of 9.5 x 1.5 cm. hundred ml medium was poured evenly in five plates and allowed to solidify for further tests.

2.1.3 Inoculation of the medium with mycelial mat

Circular discs of 10 mm diameter were cut from vigorously grown culture of *M. anisopliae* using a sterile cork borer and such discs were placed in the middle of each petriplate on the medium mixed with insecticide. Medium inoculated with the fungus without insecticide served as untreated control. These steps were carried out under aseptic conditions inside an inoculation chamber sterilized with UV radiation. These plates were incubated at $25 \pm 1^\circ\text{C}$ for 10 days.

Radial growth of the fungus was measured after 10 days and compared with untreated control. The number of conidia per unit area and viability of conidia were also recorded following the procedures mentioned in earlier experiment and compared with untreated control using the formula.

$$R = \frac{C-T}{C} \times 100$$

Where,

R = Per cent reduction of radial growth / conidia per unit area / conidial viability.

C = Radial growth / conidia per unit area / Conidial viability of fungi grown on control or untreated medium.

T = Radial growth / conidia per unit area / conidial viability of fungi grown on insecticide treated medium.

2.2 Joint Action of Microbial, Botanical and Chemical Insecticides with Selected Entomopathogenic Fungal Isolates

2.2.1 Isolation, purification, mass multiplication and maintenance of SI. NPV

Spodoptera NPV cultures were obtained from Project Directorate of Biological Control

(P.D.B.C.), Bangalore. Mass multiplication of nuclear polyhedrosis virus was done on larvae of *S. litura*. Castor leaf dipped in viral suspension of 1×10^6 PIBs/ml were fed to the third instar larvae of *S. litura* for 24 hours, later transferred to fresh semi-synthetic diet individually in glass vials. Larvae were reared on diet till development of disease. Diseased larvae were collected and stored in distilled water in 100 ml of conical flask and allowed to putrefy for 15 days. The putrefied larvae were macerated using the glass rod and then filtered through double layer muslin cloth twice. The PIBs were purified by alternate cycle of low (250-500 g rpm for 5-10 min) and high (5000-7000 g rpm for 30-60 min) speed centrifugation. The PIBs was stored at 4°C in the refrigerator for further use.

2.2.2 Mass Multiplication of standard isolate of *Bacillus thuringiensis* (HD-1)

The bacterial culture (HD-1) was collected from the Department of Plant Protection, Sam Higginbottom Institute of Agriculture Technology and sciences, Allahabad. *Bacillus thuringiensis* easily produced on artificial media by adopting conventional fermentation techniques either on surface or semi solid fermentation or submerged fermentation. strains from diseased insects is isolated as follow, the non-spore forming bacteria are eliminated by heating 60°C for 50 minutes, the growth of other spore forming is reduced by addition of $50\mu\text{g/ml}$ polymedium B to the nutrient agar medium. The slant culture of pure *Bacillus thuringiensis* (HD-1) is transferred into 300ml of polymedium (Pepton 0.5%, Glycerol 1%, Yeast extract 1%, Beef extract 0.5% and NaCl 0.3% with pH adjusted to 7.2) in 500ml flask. The incubation is done at 32°C in an incubation shaker for 48hrs. Once the incubation is completed the whole content is transferred to fermentor of 15 litres capacity containing 10 liter of presterilized poly-media. The inoculated medium is incubated in the fermentor for 72hrs. for further sealing of production 15 liter of growing culture is added to 300 litre capacity fermentor. The spore count of the fermented liquor should be 2.5×10^9 spore/ml. The content of each fermented aseptically centrifuged at 5000 rpm for 10-15 minutes. The sedimentation is washed 3 times with distilled water and transferred to small quantity of polymedium, vacuum dried and concentrated into powder and then packed.

2.2.3 Preparation of Neem seed kernel extract (NSKE-5%)

Fifty grams of neem seeds were shade dried, crushed and then soaked overnight in little quantity of water. Later, the mixture was squeezed through muslin cloth and the volume was made upto one liter so as to obtain 5% solution. The tests were conducted on the third instar larvae of *S. litura* using LC₅₀ and LC₂₅ combinations of entomopathogens and recommended dose and half recommended dose of neem oil, NSKE and spinosad (45 SC).

2.2.4 Fungus (*B. bassiana* (Bb-L-2) and *M. anisopliae* (Ma-L-1)) and *Bacillus thuringiensis* (HD-1)

Larvae of uniform age and size from the laboratory cultures were used for this study. Four combinations of concentrations were used for infecting the larvae. Healthy, third instar larvae were sprayed with conidial suspension and later, the larvae were allowed to feed on leaf treated with desired concentration of *Bacillus thuringiensis*. Observation on per cent mortality was observed daily up to ten days after the treatment.

2.2.5 Fungus *M. anisopliae* (Ma-L-1) and SI NPV

The respective conidial suspensions were sprayed on third instar larvae of *S. litura* and later allowed the larvae to feed on leaf dipped in desirable concentration of NPV. Four combination of concentration were tried using effective lethal concentration, LC₅₀ and LC₂₅.

2.2.6 *M. anisopliae* (Ma-L-1) and neem oil, NSKE and spinosad (45 SC)

The respective conidial suspensions and insecticides were sprayed on third instar larvae of *S. litura*. The following formula was used to determine expected mortality, if the two pathogens acted independently of each other.

$$E = (Ob + Os) - ((Ob \times Os) / 100)$$

Where,

E = per cent expected mortality.

Ob = Observed percentage mortality produced by one pathogen.

Os = Observed percentage mortality produced by another pathogen.

Chi-square test

$$\chi^2 = (Oc - E)^2 / E$$

Where,

Oc = Observed percentage mortality from the combination

E = per cent expected value

The calculated Chi-square value were compared to the Chi-square table value for 1 degree of freedom P=0.05. If the table value exceeded the calculated, it was concluded that the observed mortality for the combination of pathogen was within the range expected from an additive effect. If the calculated value exceeded the table value, a synergistic reaction between the pathogen was suspected [5].

3. RESULTS AND DISCUSSION

3.1 *In vitro* Compatibility of with Selected Insecticides on Radial Growth of Entomopathogenic Fungi *M. anisopliae* (Ma-L-1)

The toxic effect of six insecticides viz. Indoxacarb 14.5 SC, Spinosad 45 SC, monocrotophos 36 SL, chlorpyrifos 20 EC, dichlorvos 76 EC and Endosulfan 36 EC and two neem formulations was tested on the radial growth of the selected test strains of *M. anisopliae* (Ma-L-1). The radial growth recorded by strain Ma-L-1 on insecticides Indoxacarb 14.5 SC and Spinosad 45 SC contaminated media were 5.74 and 5.76 cm, respectively which were on par with control (5.79 cm) while monocrotophos 36 SL, chlorpyrifos 20 EC, dichlorvos 76 EC and Endosulfan 36 EC recorded 2.16, 1.59, 0 and 2.27 cm radial growth respectively, with 62.73, 72.40, 100 and 60.83 per cent growth reduction respectively, radial growth which was significantly lower compared to control. While dichlorvos 76 EC brought out 100 per cent growth reduction in compared to control (Table 1). Neem products such as neem oil and NSKE at 5% concentration caused varying level of colony inhibition of *M. anisopliae*. Neem oil affected the radial growth (14.16 % reduction) while NSKE effected the radial growth (7.77 %) (Table 1), significant reduction in radial growth of Ma-L-1 due to neem products compared with chemical insecticides was not observed. The results of the present study suggest that the insecticides Spinosad 45 SC and Indoxacarb 14.5 SC can be used with *M. anisopliae* in pest management. This combination would give an added advantage where the insecticide pathogen mixtures.

Table 1. *In vitro* compatibility of *M. anisopliae* (Ma-L-1) strain with selected insecticides

Insecticides	Concentration (%)	Radial growth (cm) after 10 days	Per cent inhibition over control
Indoxacarb 14.5% SC	0.0045	5.74 ^c	0.86
Spinosad 45% SC	0.018	5.76 ^b	0.52
Monocrotophos 36% SL	0.310	2.16 ^g	62.73
Chlorpyrifos 20% EC	0.050	1.59 ^h	72.40
Dichlorvos 75% EC (DDVP)	0.120	0 ⁱ	100
Endosulfan 36% EC	0.350	2.27 ^f	60.83
Neem oil 5%	5	3.27 ^e	43.52
NSKE 5%	5	3.74 ^d	35.40
Control	-	5.79 ^a	0
SE(m) ±		0.025	-
CD (0.01)		0.072	

Figures indicated by same letters are not significantly different from one another as per DMRT

Haseeb [6] revealed that the growth of test fungus strongly inhibited by insecticides by insecticides in descending order were chlorpyrifos, endosulfan, malathion, methyl parathion, monocrotophos and fenvalerate (72.7-94.6 % reduction in growth dia.) while dimethoate affected growth 32.3 % reduction and compatibility of six strains of *B.bassiana* with four commonly used insecticides, viz., imidacloprid, spinosad, indoxacarb and chlorpyrifos. All the strains were compatible with imidacloprid, spinosad and indoxacarb. Chlorpyrifos was found to be highly incompatible with all the strains of *B.bassiana* and exhibited high inhibition of growth [7].

3.2 *In vitro* Compatibility of Selected Entomopathogenic fungi *M. anisopliae* (Ma-L-1) with Botanicals and Chemical Insecticides against *Spodoptera litura*

3.2.1 Joint action of *M. anisopliae* (Ma-L-1) and *Sl.* NPV on third larval instar of *S. litura*

The *M. anisopliae* (Ma-L-1) and *Sl.* NPV at LC₅₀ tested against the third instar larvae of test species individually caused 47.91 cent and 57.33 per cent mortality and at LC₂₅ mortality obtained was 20.14 per cent and 27.61 per cent, respectively (Table 2).

Simultaneous exposure of *M. anisopliae* (Ma-L-1) and *Sl.* NPV at LC₅₀ each resulted in 63.81 per cent mortality. The expected mortality was 77.77 per cent. Combination of LC₅₀ of *M. anisopliae* (Ma-L-1) and LC₂₅ of *Sl.* NPV and vice versa,

showed 47.91 per cent and 51.33 per cent mortality, respectively. Mixture of *M. anisopliae* (Ma-L-1) and *Sl.* NPV at LC₂₅ each, resulted in 34.27 mortality and expected mortality for the same was 42.19 per cent (Table 3).

3.2.2 Joint action of *Sl.* NPV and *B. thuringiensis* (HD-1) on third larval instar of *S. litura*

At the higher dosage levels (LC₅₀) tested *Spodoptera* NPV and *B. thuringiensis* (HD-1) individually caused 57.33 per cent and 53.33 per cent mortality, respectively. The same pathogens at LC₂₅ showed 27.61 per cent and 25.66 per cent mortality, respectively (Table 4). The *Spodoptera* NPV and *B. thuringiensis* (HD-1) mixtures at LC₅₀ each resulted in 65.57 per cent mortality (Table 25). Combination of LC₅₀ of *Spodoptera* NPV and LC₂₅ of *B. thuringiensis* (HD-1) and vice versa resulted in 63.33 per cent and 54.45 per cent mortality. Simultaneous exposure of *Spodoptera* NPV and *B. thuringiensis* (HD-1) to test species at LC₂₅ each showed 35.26 per cent kill and the mortality expected from the same combination was 46.18 per cent.

3.2.3 Joint action of *M. anisopliae* (Ma-L-1) and *B. thuringiensis* (HD-1) on *S. litura*

At the higher dosage (LC₅₀) levels tested *M. anisopliae* (Ma-L-1) and *B. thuringiensis* (HD-1) individually caused 47.91 per cent and 53.33 per cent mortality (Table 2). The combination of these two pathogens at LC₅₀ each caused 63.33 per cent mortality (Table 5). The expected mortality from such combination was 75.68. The lower dosage levels (LC₂₅) of *M. anisopliae* (Ma-L-1)

and *B. thuringiensis* (HD-1) resulted in 20.14 per cent and 25.66 per cent, respectively. Simultaneous exposure of *M. anisopliae* (Ma-L-1) at LC₅₀ and *B. thuringiensis* (HD-1) at LC₂₅ and vice versa, resulted in 56.60 per cent and 53.33 per cent mortality, whereas the expected

mortality from such combination was 61.27 and 62.73 per cent, respectively. Combination of *M. anisopliae* (Ma-L-1) and *B. thuringiensis* (HD-1) at LC₂₅ each cause 30.00 per cent mortality, where as the expected mortality from such mixture was 40.63 per cent (Table 5).

Table 2. Percent mortality of *S. litura* larvae observed at different concentrations of selected entomopathogens and insecticides

Insecticides	Dose	% Mean mortality (after 10 days)
<i>Beauveria bassiana</i> (Bb-L-2)	LC ₅₀	45.44
	LC ₂₅	27.08
<i>Metarhizium anisopliae</i> (Ma-L-1)	LC ₅₀	47.91
	LC ₂₅	20.14
<i>Bacillus thuringiensis</i> (HD-1)	LC ₅₀	53.33
	LC ₂₅	25.66
<i>Spodoptera</i> NPV	LC ₅₀	57.33
	LC ₂₅	27.61
Spinosad (Tracer 45 SC)	RD (Recommended dose)	94.52
	1/2 RD (Recommended dose)	68.31
Neem oil	RD (Recommended dose)	34.27
	1/2 RD (Recommended dose)	20.18
NSKE (Neem seed kernal extract)	RD (Recommended dose)	29.33
	1/2 RD (Recommended dose)	17.83

B. bassiana (Bb-L-2): LC₅₀ = 5.0×10^6 conidia/ml & LC₂₅ = 2.0×10^4 conidia/ml
M. anisopliae (Ma-L-1): LC₅₀ = 1.6×10^6 conidia/ml & LC₂₅ = 1.2×10^4 conidia/ml
Bacillus thuringiensis (HD-1): LC₅₀ = 3.5×10^4 spore/ml & LC₂₅ = 2.9×10^3 spore/ml
Spodoptera NPV: LC₅₀ = 4.2×10^4 PIBs/ml & LC₂₅ = 3.3×10^3 PIBs/ml
Spinosad (Tracer 45 SC): RD (Recommended dose)=0.018% & 1/2 RD (Recommended dose)=0.009%
Neem oil: RD (Recommended dose)=5% & 1/2 RD (Recommended dose)=2.5%
NSKE (Neem seed kernal extract): RD (Recommended dose)=5% & 1/2 RD (Recommended dose)=2.5%

Table 3. Joint action of *M. anisopliae* (Ma-L-1) and *Sl.* NPV on third larval instar of *S. litura*

Combination <i>M. anisopliae</i> (Ma-L-1) + <i>Sl.</i> NPV (Concentration)	Expected per cent mortality	Per cent mortality observed	Chi - Square
LC ₅₀ + LC ₅₀	77.77	63.81	2.09
LC ₂₅ + LC ₅₀	62.29	47.91	2.46
LC ₅₀ + LC ₂₅	65.92	51.33	1.70
LC ₂₅ + LC ₂₅	42.19	34.27	1.56

Spodoptera NPV: LC₅₀ = 4.2×10^4 PIBs/ml & LC₂₅ = 3.3×10^3 PIBs/ml
M. anisopliae (Ma-L-1): LC₅₀ = 1.6×10^6 conidia/ml & LC₂₅ = 1.2×10^4 conidia/ml

Table 4. Joint action of *Sl.* NPV and *B. thuringiensis* (HD-1) on third larval instar of *S. litura*

Combination <i>Spodoptera</i> NPV + <i>B. thuringiensis</i> (HD-1) (Concentration)	Expected per cent mortality	Per cent mortality observed	Chi - Square
LC ₅₀ + LC ₅₀	80.09	65.57	2.28
LC ₅₀ + LC ₂₅	68.29	63.33	0.36
LC ₂₅ + LC ₅₀	66.22	54.45	2.09
LC ₂₅ + LC ₂₅	46.18	35.26	2.58

Spodoptera NPV: LC₅₀ = 4.2×10^4 PIBs/ml & LC₂₅ = 3.3×10^3 PIBs/ml
Bacillus thuringiensis (HD-1): LC₅₀ = 3.5×10^4 spore/ml & LC₂₅ = 2.9×10^3 spore/ml

Table 5. Joint action of *M. anisopliae* (Ma-L-1) and *B. thuringiensis* (HD-1) on *S. litura*

Combination <i>M. anisopliae</i> (Ma-L-1) + <i>B. thuringiensis</i> (HD-1) (Concentration)	Expected per cent mortality	Per cent mortality observed	Chi - Square
LC ₅₀ + LC ₅₀	75.68	63.33	2.01
LC ₂₅ + LC ₅₀	61.27	56.60	0.36
LC ₅₀ + LC ₂₅	62.73	53.33	1.41
LC ₂₅ + LC ₂₅	40.63	30.00	2.78

Bacillus thuringiensis (HD-1) : LC₅₀ = 3.5×10^4 spore/ml & LC₂₅ = 2.9×10^3 spore/ml

M. anisopliae (Ma-L-1) : LC₅₀ = 1.6×10^6 conidia/ml & LC₂₅ = 1.2×10^4 conidia/ml

The treatment involving different combinations of microbial pathogens, the Chi-square value was less than the table Chi-square. Hence, it was concluded that per cent observed mortality for the combinations were within the range expected from the additive effects. None of the combinations showed either antagonistic or synergistic effect and the results are concurrent with the earlier findings of who reported that *B. thuringiensis* and NPV combination used against the corn earworm, *Heliothis zea* proved less effective than the virus alone. However, in laboratory experiments by Chancey et al. [8] indicated that combination of NPV and *B. thuringiensis* against cabbage looper, *Trichoplusia ni* might be unsatisfactory and detrimental to the pathogenic action of the NPV. Many of these studies have involved simultaneous infection between unrelated pathogenic bacteria, fungi, microsporidian and viral interaction, [9,10] and Manjula and Padmavathamma [11] recorded no significant reduction of *M. testulalis* larvae in redgram ecosystem by combination of NPV and *B. bassiana*.

The present findings contradicted with the results reported by Bird [12] Synergistic response between insect pathogens has been reported by one of the first reports of more than one insect virus simultaneously infecting susceptible host larvae was documented, in which he described a double infection in *Choritonera fumiferana* by granulosis virus (GV) and nuclear polyhedrosis virus (NPV). Tanada, [13] reported that synergism occurred when GV and NPV of the armyworm, *Pseudaletia unipuncta* were administered together. Similarly, Lowe and Paschke [14] reported that an additive effect occurred only when a GV and NPV were simultaneously administered to the cabbage looper, *Trichoplusia ni*. Double infection by different types of pathogens in the laboratory has resulted in increased mortality [15]. Mattu and Zohdy [16] found the NPV and *B. thuringiensis* (Bactospeine) produced an antagonistic effect in

H. armigera and as the larvae increased in age, two pathogens interacted synergistically and an additive effect was observed especially when mixture contained the LC₅₀ of the bacterial pathogen.

3.2.4 Joint action of *M. anisopliae* (Ma-L-1) and selected insecticides on third larval instar of *S. litura*

The *M. anisopliae* (Ma-L-1) tested at LC₅₀ and LC₂₅ dosage and Spinosad 45 SC tested at recommended dose and half recommended dose individually caused 47.91, 20.14, 94.52 and 68.31 per cent mortality and simultaneous exposure of *M. anisopliae* (Ma-L-1) and Spinosad 45 SC against the test insect with four different dose combinations (LC₅₀ dose of *M. anisopliae* (Ma-L-1) and recommended dose of Spinosad 45 SC, LC₅₀ dose of *M. anisopliae* (Ma-L-1) and half recommended dose of spinosad 45 SC, LC₂₅ dose of *M. anisopliae* (Ma-L-1) and recommended dose of Spinosad 45 SC, LC₂₅ dose of *M. anisopliae* (Ma-L-1) and half recommended dose of spinosad 45 SC) showed 100, 100, 100 and 96.31 per cent mortality with four different dose combinations (Table 6).

M. anisopliae (Ma-L-1) tested at LC₅₀ and LC₂₅ dosage and neem oil tested at recommended dose and half recommended dose individually caused 47.91, 20.14, 34.27 and 20.18 per cent mortality and Simultaneous exposure of *M. anisopliae* (Ma-L-1) and neem oil against the test insect with four different dose combinations (LC₅₀ dose of *M. anisopliae* (Ma-L-1) and recommended dose of neem oil, LC₅₀ dose of *M. anisopliae* (Ma-L-1) and half recommended dose of neem oil, LC₂₅ dose of *M. anisopliae* (Ma-L-1) and recommended dose of neem oil, LC₂₅ dose of *M. anisopliae* (Ma-L-1) and half recommended dose of neem oil) showed 49.12, 43.27, 41.44 and 27.08 per cent mortality with four different dose combinations respectively (Table 7).

Table 6. Joint action of *M. anisopliae* (Ma-L-1) and Spinosad on *S. litura*

Combination <i>M. anisopliae</i> (Ma-L-1) + Spinosad (Tracer 45 SC) (Concentration)	Per cent mortality observed
LC ₅₀ + R.D	97.14
LC ₅₀ + ½ R.D	83.53
LC ₂₅ + R.D	95.62
LC ₂₅ + ½ R.D	74.75

M. anisopliae (Ma-L-1): LC₅₀ = 1.6×10^6 conidia/ml & LC₂₅ = 1.2×10^4 conidia/ml
 Spinosad (Tracer 45 SC) : RD (Recommended dose) = 0.018% & 1/2 RD (Recommended dose) = 0.009%

Table 7. Joint action of *M. anisopliae* (Ma-L-1) and Neem oil on *S. litura*

Combination <i>M. anisopliae</i> (Ma-L-1) + Neem oil (Concentration)	Per cent mortality observed
LC ₅₀ + R.D	49.12
LC ₅₀ + ½ R.D	43.27
LC ₂₅ + R.D	41.44
LC ₂₅ + ½ R.D	27.08

M. anisopliae (Ma-L-1): LC₅₀ = 1.6×10^6 conidia/ml & LC₂₅ = 1.2×10^4 conidia/ml
 Neem oil: RD (Recommended dose) = 5% & 1/2 RD (Recommended dose) = 2.5%

The *M. anisopliae* (Ma-L-1) tested at LC₅₀ and LC₂₅ dosage and NSKE 5% tested at recommended dose and half recommended dose individually caused 47.91, 20.14, 29.33 and 17.83 per cent mortality and simultaneous exposure of *M. anisopliae* (Ma-L-1) and NSKE against the test insect with four different dose combinations (LC₅₀ dose of *M. anisopliae* (Ma-L-1) and recommended dose of NSKE 5%, LC₅₀ dose of *M. anisopliae* (Ma-L-1) and half recommended dose of NSKE 5% LC₂₅ dose of *M. anisopliae* (Ma-L-1) and recommended dose of NSKE 5%, LC₂₅ dose of *M. anisopliae* (Ma-L-1) and half recommended dose of NSKE) showed 47.12, 44.92, 42.81 and 33.08 per cent mortality with four different dose combinations respectively (Table 8).

Similar findings of synergism of microorganism and chemical insecticides have been very well documented [17,18] and Wang et al. [19]. Similar findings of compatibility of insecticides with

various entomopathogens was reported by Fargues [20] who reported that combinations of sublethal amount of insecticides were clearly compatible with *B. bassiana* and use of mixtures might have advantages for Colorado potato beetle management and Georghiou [21] reported that insecticide-pathogen combinations introduce multiple mortality factors against the pest and increasing the number of mortality factors used against insect and should delay any expression of resistance to new insecticides. Anderson et al. [22] reported that in bioassay with neonate of Colorado potato beetle, effects *B. bassiana* alone were extremely variable and combination of *B. bassiana* with insecticides viz. Thuringiensin, Abamectin and Triflamuron were consistently more toxic than *B. bassiana* and Sinha [23], reported that a water dispersible powder formulation of neem product (Achook) checked the larval and pupal survival and growth and adult emergence of *H. armigera*. and Ingle et al. [24] reported that effectiveness of

Table 8. Joint action of *M. anisopliae* (Ma-L-1) and NSKE on *S. litura*

Combination <i>M. anisopliae</i> (Ma-L-1) + NSKE (Concentration)	Per cent mortality observed
LC ₅₀ + LC ₅₀	47.12
LC ₅₀ + ½ R.D	44.92
LC ₂₅ + LC ₅₀	42.81
LC ₂₅ + ½ R.D	33.08

M. anisopliae (Ma-L-1): LC₅₀ = 1.6×10^6 conidia/ml & LC₂₅ = 1.2×10^4 conidia/ml
 NSKE (Neem seed kernal extract) : RD (Recommended dose) = 5% & 1/2 RD (Recommended dose) = 2.5%

entomogenous fungus, *Nomuraea rileyii* with combination of different plant oils on chickpea against *Helicoverpa armigera*, sprayings of soybean and sunflower oil formulation combinations were found very effective in reducing larval population, pod damage and increase in grain yield of chickpea.

4. CONCLUSION

The present study showed that EPF, *Metarhizium anisopliae* can help to delay the onset of resistance and is most virulent against the *Spodoptera litura*. Furthermore, the environmental hazards of pesticides demand some eco-friendly and bio-rational alternatives. Application of *Metarhizium anisopliae* would be a better choice instead of insecticides while dealing resistance. Based on the assumptions to be tested, EPF seem to be the best choice and successful alternatives for alleviation of the pesticide resistance problem and hence achieving the goal of increased productivity. It can be concluded that integration of entomopathogenic fungi and insecticides had an advantage for pest management. Actual effects on the fungi at cellular as well as field level need to be investigated to understand if the effects are permanent or temporary.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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