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Research Article

Combined Efficacy of Biorational Insecticides against Potato Leafworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) Under Laboratory and Field Conditions

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AS and TNS planned and executed the work. AS and HH wrote the manuscript. HM helped in experiments. MJ, SS and TN collected the smaples and conducted the experiments. TNS, AS and HH supervised the study. TNS proofread the manuscript.

Keywords
Spodoptera litura, Integrated pest management, Synthetic pesticides, Botanical formulations, Binary combinations

Abstract | Armyworm species *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is one of the destructive polyphagous insect pests worldwide and has attained field-evolved resistance to most of the conventional synthetic insecticides. In this study, some previously selected most effective biorational synthetic, botanical and microbial insecticidal formulations were evaluated either alone or in binary combinations against 3rd instar larvae of *S. litura* under laboratory and then under the field conditions. In both trials, insecticidal treatments affected significantly the mean mortality or reduction of *S. litura* larvae recorded both at two and five days post-treatment. In laboratory bioassay, combinations of flubendiamide and *A. indica*, flubendiamide and *N. tabacum* and of spinetoram and *A. indica* formulations caused significantly high mortality (100%), followed by *B. thuringensis* and *S. litura*-NPV (94.92%) and *A. indica* and *B. thuringensis* (93.22%) and exhibited a synergized toxicity against *S. litura* larvae as compared to other treatments. In two years field trials, binary combination of flubendiamide and spinetoram showed an average larval reduction of 59 – 100%, followed by 100% larval reduction exhibited by flubendiamide and spinetoram alone and by the combination of flubendiamide and *A. indica* formulations at 5th day of application. While, minimum larval reduction was recorded for both microbial insecticides alone and in combination for both years. Overall results of this *in-vitro* and *in-situ* evaluation demonstrate the effectiveness of biorational insecticidal formulations and recommend their incorporation in integrated management of *S. litura*.

Novelty Statement | Laboratory and then field evaluations of some promising botanical, microbial and non-conventional synthetic insecticidal formulations against 3rd instar larvae of armyworm (Spodoptera litura) comprise the novelty of this study.

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Introduction

Armyworm *Spodoptera litura* Fabricius is a destructive noctuid pest infesting a wide range of crops including vegetables, fruits and agronomic crops world

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wide including Indo-Pak region (Bragard et al., 2019). It infests and causes substantial damage (up to 100%) to many ornamental and agronomic crops including maize, brassica, wheat, cotton, potato and gram (Ahmad et al., 2013; Batool et al., 2022). *S. litura* is being emerged as a challenging pest of potato crop in Pakistan for last few years causing substantial damage to potato foliage resulting in considerable qualitative and quantitative loss to potato crop (Ahmad et al., 2013, 2021).

Indigenous potato growers primarily depend on blind and extensive use of highly broad-spectrum and persistent synthetic insecticides against infestations of *S. litura*. Still, it has been a difficult to control pest because of high incidence of resistance it exhibits against most of the conventional synesthetic insecticides being used by the farmers (Saleem et al., 2016; Zhang et al., 2022). Apart from this insecticide resistance problem, there are other ecological consequences of wide use of conventional synthetic insecticides such as contamination of environment, suppression of non-target species including insect pests' natural enemies and human health (Serrão et al., 2022).

This situation demands for seeking some alternate environment-friendly pest control techniques for instance microbial, botanical, and non-conventional synthetic insecticidal formulations which are more biorational and are less toxic to non-target fauna (Arthurs and Dara, 2019; Rani et al., 2021; Acheuk et al., 2022; Ahmed et al., 2022). To this end, this study tested some selected biorational insecticidal formulations including botanical, microbial and differential chemistry non-conventional synthetic pesticides against 3rd instar laboratory-reared *S. litura* first under the lab conditions and then the most effective treatments were further tested against 3rd instar larvae either alone and/or in binary combinations in laboratory and under field conditions. The primary objective of this research work was to have the comparative evaluation of binary combinations of some already screened out most effective biorational insecticidal formulations including two botanical formulations (*i.e. Azadirachta indica* oil and *N. tabacum* extract), two microbial insecticides (*i.e. Bacillus thuringensis kurstaki* and *S. litura*–NPV) and two non-conventional synthetic insecticides (*i.e.* flubendiamide and spinetoram) against *S. litura* 3rd instar larvae under *in-vitro* and *in-situ* setups. These six insecticidal formulations were selected from a previous laboratory screening.

Materials and Methods

Insects rearing

Late 4th or 5th instar larvae of *S. litura* were sampled from the potato crop located in the surroundings of district Lahore (31°34'55.5" N; 74°10'8.2" E), and were shifted to the laboratory of the Department of Entomology, College of Agriculture, University of Sargodha, for further rearing on artificial (chickpea powder-based) diet prepared after Jin et al. (2020). Culture of armyworm was reared for many generations prior to its utilization in bioassays. Rearing was done at 65±5 % relative humidity and at 26 ± 2°C temperature under 14:10 h light: dark photoperiod. In all experiments, healthy and active early freshly molted 3rd instar larvae were used.

Insecticidal treatments

In a previous laboartory study (Ahmad et al., 2023), we screened out ninteen biorational non-conventional synthetic, microbial and botanical insecticides against 3rd instar *S. litura*. Six insecticidal formulations, as described in Table 1, were selected from this study for their further evaluation under laboratory and field conditions. These insecticidal treatments were comprised of two most effective synthetic insecticides (flubendiamide (Belt®, Bayer) and Spinetoram (Radiant®, Dow AgroSciences™), two effective botanical (Nicotine 10% EC (*Nicotiana tabacum*) and Neem oil 0.3% EC (*Azadirachta indica*), and two microbial (*Bacillus thuringensis* var *kurstaki* (Lipel® AgriLife™) and *Spodoptera litura*–NPV (Somstar® AgriLife™) insecticidal formulations. LC_{25} values of these insecticidal treatmetns as given in Table 1 were used for their independent and binary evaluations against 3rd instar larvae of *S. larvae* under both laborary and field conditions.

Table 1: Selected biorational synthetic, botanical and microbial insecticides bioassayed alone and in binary combinations against 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatment code	Treatment	Mode of Action*	LC_{25} Used
I1	Flubendiamide	Ryanodine receptor modulator	390 ppm
I2	Spinetoram	N-acetyl cholinesterase (nAChR) allosteric modulator	625 ppm
M1	*Bacillus thuringensis* kurstaki	δ-endotoxin-induced septicemia	6.10×10^6 spores mL^{-1}
M2	*Spodoptera litura*–NPV	Virions-induced septicemia	1.89×10^3 OB mL^{-1}
B1	*Azadirachta indica* oil	Azadirachtin-induced ecdysteroids disruption and antifeedant	19 ppm
B2	*Nicotiana tabacum* oil	Nicotinic acetylcholine receptor (nAChR) competitive modulators	92.5 ppm

*According to Insecticide Resistance Action Committee (www.irac-online.org) IRAC MoA Classification Version 9.4, June 2022. OB, occlusion bodies.

Toxicity bioassays in laboratory

Toxicological bioassays with LC_{25} of insecticidal formulations were performed in the laboratory using previously described protocols by Nathan and Kalaivani (2006), Enriquez et al. (2010) and Paul and Chaudhary (2016) after slight modifications. Completely randomized design (CRD) was followed for all laboratory trials with 8–10 replications for each treatment in sterilized plastic Petri-plates (dimensions: 60×15mm). Insecticidal solutions were made using distilled water and were sprayed on foliage of potted potato plants (cultivar Diamant) using hand-held atomizers (50 mL) and their leaf discs (diameter: 60 mm) were prepared and lined on 1.0% agar layer in Petri-plates. Freshly molted 3rd instar healthy and active larvae of laboratory reared *S. litura* larvae were released on these Petri-plates (10 larvae per plate) and were placed in the incubator (Sanyo MLR-350H, Sanyo, Japan) set at 65% ± 5 relative humidity, 26 ± 2°C, and at photoperiod 14:10 h light: dark. Leaf discs were replaced at alternate days during incubation. Mortality of larvae was observed at 2 and 5 days post-exposure.

Field evaluation of insecticides

For *in-situ* evaluation of the most effective insecticidal formulations or of their binary combinations, potato plants (cultivar: Diamant) were sown on ridges using 45 and 25 cm row-to-row and plant to plant distance, respectively. Experimental plan was as per randomized complete block design (RCBD). Size of the experimental plot was 5 × 5 ft and each treatment was replicated thrice. Five early 3rd instar laboratory reared larvae were released and allowed to settle on each plant and next day (after 24 h) the insecticidal treatments were sprayed on plant foliage using manual spray bottles. Data on larval count were collected at 2 and 5 days of insecticidal applications.

Statistical analysis

Data regarding larval mortality or larval reduction in case of field trial were presented graphically and were statistically analyzed by Statistix® Version 10.0 (Analytical Software, Tallahassee, Florida). Before statistical analysis, Abbott's formula (Abbott, 1925) was employed to correct the mortality data. One-way ANOVA (analysis of variance) followed by Tukey's HSD (highly significant difference) post-hoc test at 95% level of significance was used for statistical analysis of larval mortality data. While two-way ANOVA was used for the analysis of larval reduction data followed by Fisher's LSD (least significant difference) post-hoc test at 95% level of significance.

Results

Combined toxicity of biorational insecticides against 3rd instar S. litura larvae

First of all, LC_{25} of selected most effective biorational pesticides comprising of two botanical, two synthetic and two microbial insecticidal formulations were bioassayed alone and in different binary mixtures against 3rd instar *S. litura* larvae using leaf-disc dip method. According to the factorial analysis of variance, all insecticidal treatments exerted a significant impact on the mean larvae mortality observed both at 2 days post-exposure ($F_{20, 105}$ = 29.08; P = < 0.001) and at 5 days of exposure ($F_{20, 105}$ = 56.43; P = < 0.001) (Table 2).

At 2 days post-exposure, significantly high larval mortality (96.67%) was exhibited by the combination of flubendiamide and *A. indica* formulations, followed by the combined treatments of spinetoram + *A. indica* (768.33%) and flubendiamide + spinetoram (73.33%). Binary combinations of spinetoram + *N. tabacum*, *B. thuringensis* + *N. tabacum* and *B. thuringensis* + *S. litura*-NPV showed minimum mortality (35.0 – 36.67%). Least effective treatments were *N. tabacum*, spinetoram and *S. litura*-NPV alone exhibiting minimum (10 – 20%) larval mortality (Figure 1).

According to observation made at 5 days post-treatment, combinations of flubendiamide + *A. indica*, flubendiamide + *N. tabacum* and of spinetoram + *A. indica* formulations caused highest and significant mortality (100%) followed by *B. thuringensis* + *S. litura*-NPV (94.92%) and *A. indica* + *B. thuringensis* (93.22%). Among binary combinations, spinetoram + *N. tabacum* and *S. litura*-NPV + *N. tabacum* showed minimum larval mortality (47.46-52.54%). While minimum larval mortality (23.73-57.63%) was recorded for all insecticidal treatments alone at 5th day of bioassay (Figure 2).

Table 2: Analysis of variance comparison table for the mean percent mortality of 3rd instar larvae of *Spodoptera litura* exposed to different biorational synthetic, botanical and microbial insecticides alone and in binary combinations under laboratory conditions.

Source	DF	2 days post-exposure				5 days post-exposure			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value
Treatment	20	51515.9	2575.79	29.08	< 0.001	66696.6	3334.84	56.43	< 0.001
Error	105	9300.3	88.57			6205.7	59.10		
Grand mean	46.03					70.47			
CV	20.45					10.91			

P < 0.001 (highly significant) and *P* < 0.01 (significant); two-way factorial ANOVA at α = 0.05.

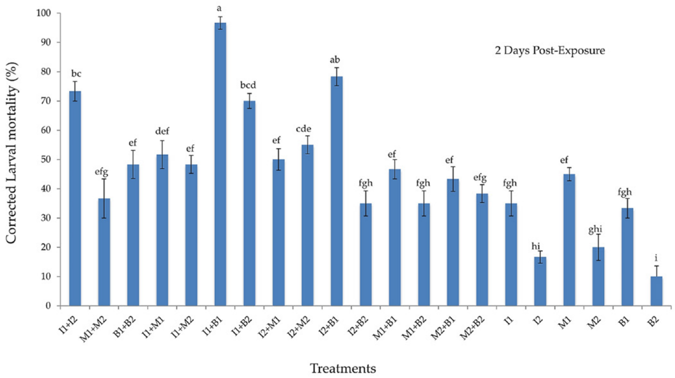

Figure 1: Corrected percent mortality (mean ± S.E.; N = 6) of 3rd instar larvae of *Spodoptera litura* bioassayed against different biorational synthetic, botanical and microbial insecticides alone and in binary combinations. I1= flubendiamide, I2= spinetoram, B1= *Azadirachta indica* oil, B2= *Nicotiana tabacum* oil, M1= *Bacillus thuringensis kurstaki*, M2= *Spodoptera litura*–NPV. Alphabets at bar tops indicate significant difference among the treatments (one-way factorial ANOVA followed by Tukey's post-hoc test HSD at α= 0.05).

Moreover, among all binary combinations of the insecticidal formulations tested, eight combinations (*i.e.* spinetoram + *A. indica*, flubendamide + *N. tabacum*, spinetoram + *S. litura*-NPV, flubendamide + spinetoram, flubendamide + *A. indica*, spinetoram + *N. tabacum*, *S. litura*-NPV + *N. tabacum* and *A. indica* + *N. tabacum*) exibited synergized toxicity (having combination factor > 1.0) against 3rd instar *S. litura* larvae under lab conditions,while remaining combinations showed antergistic effect having factor < 1 at 2 days post-treatment. Similarly, at 5 days post-treatment, only five combinations (*i.e.* spinetoram + *A. indica*, flubendamide + *B. tabacum*, spinetoram + *S. litura*-NPV, flubendamide + spinetoram and flubendamide + *A. indica*) showed a synergistc effect against the larvae of *S. litura* (Table 3).

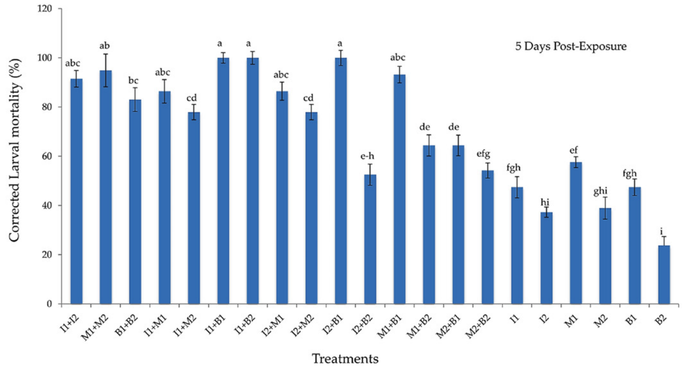

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Table 3: Effect of binary combinations of different selected biorational synthetic, botanical and microbial insecticides on 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatments	Actual mortality		Expected mortality		Factor		Effect	
	2 DPE	5 DPE	2 DPE	5 DPE	2 DPE	5 DPE	2 DPE	5 DPE
I2+B1	78.33	100.00	50.00	84.75	1.57	1.18	Synergy	Synergy
I1+B2	70.00	100.00	45.00	71.19	1.56	1.40	Synergy	Synergy
I2+M2	55.00	77.97	36.67	76.27	1.50	1.02	Synergy	Synergy
I1+I2	73.33	91.53	51.67	84.75	1.42	1.08	Synergy	Synergy
I1+B1	96.67	100.00	68.33	94.92	1.41	1.05	Synergy	Synergy
I2+B2	35.00	52.54	26.67	61.02	1.31	0.86	Synergy	Antergy
M2+B2	38.33	54.24	30.00	62.71	1.28	0.86	Synergy	Antergy
B1+B2	48.33	83.05	43.33	71.19	1.12	1.17	Synergy	Synergy
I1+M2	48.33	77.97	55.00	86.44	0.88	0.90	Antergy	Antergy
M2+B1	43.33	64.41	53.33	86.44	0.81	0.75	Antergy	Antergy
I2+M1	50.00	86.44	61.67	94.92	0.81	0.91	Antergy	Antergy
I1+M1	51.67	86.44	80.00	105.08	0.65	0.82	Antergy	Antergy
M1+B2	35.00	64.41	55.00	81.36	0.64	0.79	Antergy	Antergy
M1+B1	46.67	93.22	78.33	105.08	0.60	0.89	Antergy	Antergy
M1+M2	36.67	94.92	65.00	96.61	0.56	0.98	Antergy	Antergy

I1 = flubendiamide, I2 = spinetoram, B1 = *Azadirachta indica* oil, B2 = *Nicotiana tabacum* oil, M1 = *Bacillus thuringensis* kurstaki, M2 = *Spodoptera litura*–NPV, DPE = days post-exposure. Synergistic or antagonistic effect of binary mixtures of insecticides was determined by dividing the actual mortality of mixture with the expected mortality of both treatments alone. If the factor is less than 1.0, it was considered as Antergy and if it is more than 1.0, the effect was considered as synergy.

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Effect of insecticidal formulations on S. litura larval population under field conditions

In case of field evaluation of the most effective insecticidal formulations alone and as effective combinations screened out from laboratory bioassays, all insecticidal treatments and time factor and their interactions exerted a significant effect on the larval reduction for both years of experiment (Table 4). In winter 2019, maximum larval reduction was recorded for the potato plots treated with flubendiamide + spinetoram (100%), followed by the combination of *N. tabacum* + flubendiamide (95%), while *B. thuringensis* and *S. litura*-NPV exhibited minimum reduction alone and in combination (*i.e.,* 40 – 55%) and M2 (55%) treated plots showed minimum larval reduction after 5 days of application (Figure 3).

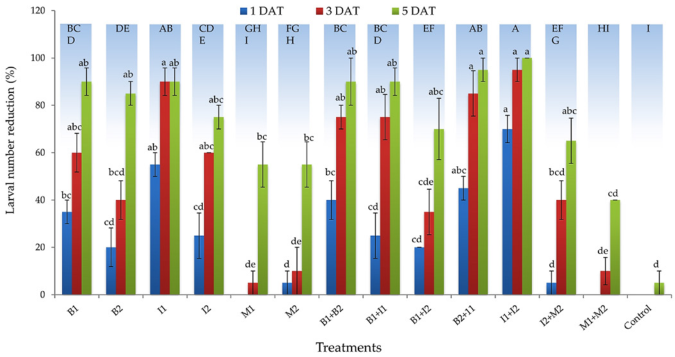
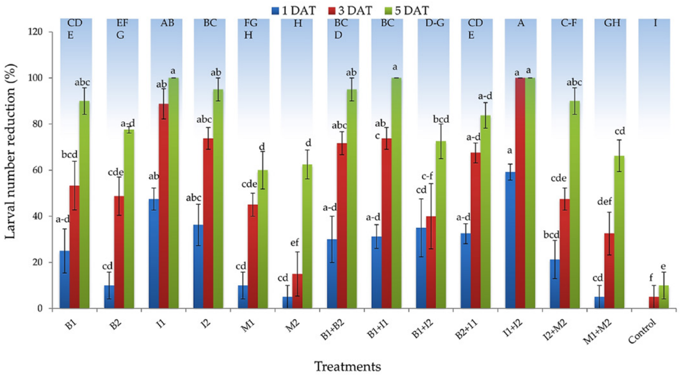


Figure 3: Percent reduction (mean ± S.E.; N = 4, n = 40) in *Spodoptera litura* larval numbers on the potato plants recorded at different time intervals post-treatment by different biorational synthetic, botanical and microbial insecticides alone and in binary combinations in winter 2019. I1 = flubendiamide, I2 = spinetoram, B1= *Azadirachta indica* oil, B2 = *Nicotiana tabacum* oil, M1 = *Bacillus thuringensis kurstaki*, M2 = *Spodoptera litura*–NPV. Small and capital alphabets indicate significant difference among the treatments for each DAT and overall among the treatments, respectively (factorial ANOVA followed by Tukey's post-hoc test HSD at α= 0.05).

Similar trend of efficacy was recorded for 2[nd] year repetition of the trial in winter 2020. Combination of synthetic insecticides flubendiamide and spinetoram showed 59-100% larval reduction from 1[st] to 5[th] day post-application, followed by 100% larval reduction exhibited by flubendiamide and spinetoram alone and by combination of flubendiamide + *A. indica* formulations at 5[th] day of application. While, minimum larval reduction was recorded for both microbial insecticides either alone and in combination (Figure 4). Negligible larval reduction (0-10%) was recorded in control plots for both year field trials.



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Discussion

Potato is an important vegetable crop of Pakistan having a substantial share both in terms of area and production. For last few years, local potato growers are being challenged by the attack of crop foliage by

Table 4: Analysis of variance comparison table for the mean percent mortality of 3[rd] instar larvae of *Spodoptera litura* exposed to different biorational synthetic, botanical and microbial insecticides alone and in binary combinations under field conditions.

Source	DF	Winter 2019				Winter 2020			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value
Treatment	13	105933	8148.7	43.51	< 0.001	72755	5596.5	35.63	< 0.001
Time	2	62233	31116.7	166.13	< 0.001	81607	40803.5	259.81	< 0.001
Treatment × Time	26	12967	498.7	2.66	0.002	10989	422.6	2.69	< 0.01
Replication	3	162	54.0			2876	958.8		
Error	123	23038	187.3			19317	157.1		
Grand mean	48.33					52.69			
CV	28.32					23.79			

P < 0.001 (highly significant) and *P* < 0.01 (significant); two-way factorial ANOVA at α = 0.05.

armyworm *S. litura*. It has become a difficult to control pest due to its field-evolved resistance against prevailing old-chemistry synthetic pesticides (Ahmad et al., 2013; Saleem et al., 2016; Zhang et al., 2022). Therefore, use of reduced-risk biorational insecticides such as non-conventional differential-chemistry synthetic, botanical and microbial insecticidal formulations would be effective to combat *S. litura* infestations on potato crop with an improved potato quality and minimized ecological risks associated with conventional synthetic pesticides.

In a previous study (Ahmad et al., 2023), a comparative evaluation of lethal toxicity and sublethal effects of some selected biorational insecticidal formulations was done against 3rd instar *S. litura* larvae and found two synthetic, two botanical and two microbial insecticidal formulations (as described in Table 1) as the most effective treatments against *S. litura* larvae. In this study, we further tested the LC_{25} concentrations of all these insecticidal treatments either alone or in combinations against 3rd instar larvae first under laboratory and then under field conditions.

In case of laboratory bioassays, both synthetic insecticides (flubendiamide and spinetoram) and botanical formulations *(A. indica* and *N. tabacum*) showed a synergized toxicity against 3rd instar *S. litura* larvae under lab conditions. Both microbial insecticides (*B. thuringiensis* and *S. litura*-NPV) either showed no additive effect or antagonized the toxicity when applied in combination with synthetic and botanical insecticides.

Our results corroborate the results of previous studies demonstrating significant toxicity of botanical pesticides including neem (*A. indica*) and tobacco (*N. tabacum*) extracts and of non-conventional synthetic insecticides including flubendiamide and spinetoram against different armyworm and other lepidopterous pest species (Nagal and Verma, 2015; Liu et al., 2017; Ayyub et al., 2019; Duarte et al., 2019; Thakur and Srivastava, 2019; Dáder et al., 2020; Phambala et al., 2020; Kong et al., 2021; Hernandez-Trejo et al., 2021; Thakur et al., 2022).

Regarding field evaluation of the most effective insectcides, in winter 2020, after five days of application, treatments flubendamide + spinetoram, *A. indica* + flubendamide and flubendamide alone gave maximum cumulative larval reduction (100%), followed by spinetoram alone (95%) and *A. indica* + *N. tabacum* (95%), while *B. thuringensis* kustaki and *S. litura*-NPV revealed minimum larval reduction *i.e.* 60 and 62.5%, respectively. During both years of the field trial, synthetic insecticides flubendiamide, spinetoram and botanical formulations of *A. indica* (neem) oil and (tobacco) *N. tabacum* appeared most effective and significantly reduced the *S. litura* larval populations in both seasons. While both microbial insecticidal treatments exhibited 50–60% larval reduction on 5th day of observation.

Although most of the insect hosts become dead within few days by the bacterial or viral induced spectcima (Lacey, 2017; Soumia et al., 2021) and the observation at 5th day of bioassay or microbial exposure was enough to see if these are effective against the *S. litura* larvae. Both microbial formluations (*B. thuringiensis* kustaki and *S. litura*-NPV) exhibited minimum toxicity in lab and were also the least effective as well under field conditons. This might be because of the limited compatibility of particular entomopathogenic strains used in the microbial formulations against the larval strain of *S. liutra* tested in the study (Maistrou et al., 2020).

Conclusions and Recommendations

In brief, this laboratory study revealed the effectiveness of aforementioned botanical and non-conventional synthetic pesticides against 3rd instar *S. litura* larvae, and advocates recommendation and potential incorporation of binary combinations of these biorational pesticides in integrated control of *S. litura* and other lepidopterous pests on vegetable crops. However, future perspectives of this study constitute the evaluation of the non-target effects of these effective insecticidal treatments on beneficial organisms including insect natural enemies (such as local predators and parasitoids of *S. litura*).

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Conflict of interest

The authors have declared no conflict of interest.

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