Pakistan J. Zool., pp 1-13, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20221026081016

Laboratory Evaluation of Selected Biorational Insecticidal Formulations against Potato Leafworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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Article Information
Received 26 October 2021
Revised 18 November 2022
Accepted 11 December 2022
Available online 19 May 2023
(early access)

Authors' Contribution
MZM and MA conceived and designed the experimental protocols. MSA, MA and HS performed the experiments and recorded data. MSA and AM performed statistical analyses. MSA, HS and MZM prepared the manuscript. MA and SI provided technical assistance in experimentation. MZM, AM and MA performed technical proofreading of the manuscript.

Key words
***Spodoptera litura*, Integrated pest management, Biorational pesticides, Botanical formulations, Microbial insecticides, Sublethal effects**

ABSTRACT

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is a deleterious agricultural pest worldwide. Field populations of *S. litura* manifest resistance to almost all conventional insecticides and it is imperative looking for novel biorational insecticides to control this pest. In this regard, this study bioassayed some promising biorational insecticides including botanical, microbial and non-conventional synthetic insecticidal formulations against 3rd instar larvae of *S. litura*. Bioassay with botanical formulations showed a significant toxicity of oil and extract formulations of neem (*Azadirachta indica*) causing 70–77% larval mortality in 72 h observation and exhibiting minimum medial lethal concentration (LC_{50}) and time (LT_{50}) values (*i.e.* 12.32 and 38.01 ppm and 16.67 and 11.68 days, respectively). Among microbial formulations tested, *S. litura*-nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis kurstaki* appeared as the most effective microbial treatments exhibiting minimum LC_{50} (3.78×10^3 OB mL^{-1} and 1.22×10^7 spores mL^{-1}, respectively) and LT_{50} (3.83 and 3.71 days, respectively) values. While flubendiamide, chlorantraniliprole and spinetoram exerted most significant lethal and sublethal effects on *S. litura* with minimum LT_{50} values (*i.e.* 19.58, 30.78 and 26.25 h, respectively). Larval development time was significantly prolonged by both half and one-fourth doses of flubendiamide and chlorantraniliprole (19.51 and 19.63 days and 17.77 and 17.20 days, respectively), while pupal duration prolonged for spinetoram and lufenuron. Similarly, significant suppression of adult lifespan was exhibited by flubendiamide (11.83 and 11.85 days) and chlorfenapyr (12.28 and 12.06 days). Overall study results advocate further consideration of these aforesaid biorational insecticides against *S. litura* infestations. However, assessment of their compatibility with each other and with other IPM strategies both under lab and field conditions constitutes future perspectives of this work.

INTRODUCTION

Armyworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is an economically important pest of a wide array of horticultural and agricultural crops worldwide (Abdullah *et al*., 2019; Bragard *et al*., 2019). In Asia-pacific region including Pakistan, this species attacks and causes substantial damage (5–100%) to various field crops including cotton, maize, wheat, gram, brassica, potato etc. (Oerke *et al*., 1994; Ahmad *et al*., 2013). For last few years, *S. litura* has been emerging as a deleterious pest of potato crop in Pakistan. It causes considerable damage to foliage of potato crop resulting in significant quantitative and qualitative yield loss (Ahmad *et al*., 2013). Most of the indigenous potato growers rely primarily on extensive and recurrent applications of highly persistent and broad-spectrum synthetic insecticides to combat *S. litura* infestations. However, it appears as a

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0030-9923/2023/0001-0001 $ 9.00/0

difficult to control pest due to high incidence of insecticide resistance in field populations of *S. litura* (Shad *et al*., 2012; Tong *et al*., 2013; Saleem *et al*., 2016; Zhang *et al*., 2022). Moreover, the disruption of non-target fauna including insect predators and parasitoids, resurgence and outbreaks of secondary pests and environmental contaminations posing human health hazards are being manifested by this excessive reliance on conventional synthetic insecticides (Khan and Ahmad, 2019; Serrão *et al*., 2022). These aforementioned ecological consequences of conventional synthetic insecticides necessitate looking for alternate biorational pest control strategies such as botanical, microbial and differential-chemistry synthetic insecticides which would be environmentally benign and less toxic to non-target fauna (Granados-Echegoyen *et al*., 2021; Rani *et al*., 2021). Development and evaluation of such biorational pesticides have been a focal point of modern plant protection research. A wide number of biopesticides have been effectively demonstrated against different sucking and chewing insect pests and most of these insecticides are biorational exhibiting less mammalian toxicity and environmental persistence and more target specificity and biodegradability (Ishaaya and Degheele, 1998; Lacey, 2017; Qadir *et al*., 2021; Acheuk *et al*., 2022).

Many plant-derived compounds including phytoextracts and essential oils are being used against various insect pest species of field crops and stored grain insect pests (Isman, 2015, 2020; Ahmed *et al*., 2022; Landero-Valenzuela *et al*., 2022). Similarly, microbial insecticides including entomopathogenic fungi, nematodes, bacteria and viruses are being researched and commercially used against different insect pests (Rai *et al*., 2014; Ullah *et al*., 2022). Similarly, synthetic insecticides with novel modes of action and chemistry such as diamides, fenoxycarbs, pyrroles, benzoylurea, avermectins and spinosyns etc. have been shown very effective for the management of resistant insect pests (Ahmad and Gull, 2017; Sparks *et al*., 2020; Idrees *et al*., 2022). A number of studies have reviewed the effectiveness of these botanical and microbial pesticides against a wide array of insect pests both under laboratory and field conditions (Copping and Menn, 2000; Arthurs and Dara, 2019; Yasin *et al*., 2020; Mansour and Biondi, 2021; Narciso *et al*., 2021; Duso *et al*., 2022).

In view of the aforesaid, this research work was aimed to assess the comparative toxicity and effectiveness of 19 promising botanical, microbial and differential-chemistry synthetic insecticides against 3rd instar larvae of laboratory reared *S. litura* with ultimate objective to find out the most effective biorational insecticidal treatments which can be recommended to farmers combating *S. litura* infestations on potato and other vegetable crops. We hypothesized that *S. litura* larvae would be highly susceptible to these non-conventional biorational insecticides including six botanical formulations (*i.e*. neem extract, nicotine, pyrethrin, neem oil, rotenone and matrine), six microbial insecticides (*i.e*. *Bacillus thuringensis* var. *kurstaki*, *Beauveria bassiana*, *Isaria fumosorosea*, *Metarhizium anisopliae*, *S. litura* NPV and *Verticillium lecanii*) and seven non-conventional synthetic insecticides (*i.e*. chlorantraniliprole, chlorfenapyr, fenoxycarb, flubendiamide, methoxyfenozide, lufenuron and spinetoram).

MATERIALS AND METHODS

Insect culture

Late instar larval population of *S. litura* was collected from the potato field (31°33'1.2" N; 74°13'19" E) and were brought to the laboratory for further rearing on artificial semi-synthetic (chickpea-based) diet prepared after slight modifications of protocol described by Jin *et al*. (2020). Insects were reared for at least three generations prior to their utilization in the experimentation. Rearing conditions were maintained at 25 ± 2°C, 70% ± 5 relative humidity and 14:10 hours light: dark photoperiod. As for laboratory bioassays involving the insecticidal evaluations against lepidopterous pests, usually early 3rd instar larvae are used because these are easy to handle or manipulate during the experimentation and are susceptible enough to respond to different insecticidal treatments than the other larval instars. In fact, 1st or 2nd instar larvae are delicate and soft and are vulnerable to mechanical damage while manipulating / handling, while later (4th-6th) instar larvae are somewhat resistant and do not respond well to treatments. Therefore, only early (freshly molted) 3rd instar larvae were used in all bioassays in this study.

Insecticidal treatments

Selected insecticidal products were procured from the authorized pesticide dealers and distributors from the grain market of Lahore (Punjab, Pakistan). Plant-based insecticidal products (botanicals) included Matrine 0.6% EC (*Sophora flavescens*), Pyrethrin 5.0% OL (*Chrysanthemum cinerariaefolium*), Rotenone 7.5% EC (*Derris* spp.), Nicotine 10% EC (*Nicotiana tabacum*), Neem oil 0.3% EC and extract 2.0% SL (*Azadirachta indica*) procured from Kingbo Biotech Co., Ltd, Beijing, China. While selective differential-chemistry synthetic insecticides included chlorantraniliprole (Coragen®, DuPont™), chlofenapyr (Pirate®, Swat Agro Chemicals), fenoxycarb (Insegar®, Syngenta Pakistan), flubendiamide (Belt®, Bayer), lufenuron (Match®, Syngenta Pakistan),

methoxyfenozide (Runner®, Arysta Life Science) and Spinetoram (Radiant®, Dow AgroSciences™). Details of all these insecticidal products evaluated in the study are provided in Tables I and II. Microbial formulations included *Bacillus thuringensis* var *kurstaki* (Lipel® AgriLife™); *Beauveria bassiana* (Racer® AgriLife™); *Verticillium lecanii* (Mealikil® AgriLife™); *Isaria fumosoroseus* (AgriLife™); *Metarhizium anisopliae* (Pacer® AgriLife™) and *Spodoptera litura*–NPV (Somstar® AgriLife™).

Toxicity bioassays with botanical insecticides

For toxicological bioassays conducted with botanical formulations, method as described by Paul and Chaudhary (2016) was used after slight modifications using plastic Petri-plates (dimensions: 60 × 15 mm). Potato plants (seed potatoes of cultivar diamant) used in the bioassays were procured from the Potato Research Institute, Sahiwal, Pakistan. Bioassays were laid out according to completely randomized design (CRD) with 10 replications for each treatment. Based on preliminary trials, five different ppm concentrations of each botanical formulation causing 10–90% larval mortality (as described in Table I) were prepared using distilled water and were applied on potted potato plants using manual spray bottles. Discs (diameter: 60 mm) of fresh potato leaves from treated and untreated potted plants were prepared and were placed in sterilized Petri-plates pre-lined with 1.0% agar medium, and ten early 3rd instar larvae of *S. litura* per plate were released on these discs.

These petri-plates were then incubated in an environment chamber (Sanyo MLR-350H, Sanyo, Japan) set at 25 ± 2°C, 70% ± 5 RH and 14:10 h light: dark photoperiod. For lethal toxicity, larval mortality was recorded at regular time intervals (6, 12, 24, 48 and 72 h post-exposure). For the determination of repellency potential of botanicals, choice test was used. In brief, one 3rd instar larva was exposed to two halves of a potato leaf disc (diameter: 60 mm) fixed apart in a Petri-plate (diameter: 60 mm). One half was treated with LC_{10}, LC_{30} or LC_{50} of each botanical and one was treated with water (control). Presence or feeding activity of larva on these leaf discs was observed at 6 and 12 h post-exposure (Lo Pinto *et al*., 2022). Ten independent replications were maintained for each treatment.

Pathogenicity bioassays with microbial formulations

Evaluation of selected microbial insecticides was done following a previously described protocol (Nathan and Kalaivani, 2006) after slight modifications. Bioassays were laid out according to completely randomized design (CRD) with 10 replications for each treatment. Entomopathogens were purified and mass-cultured from the commercial formulations and then using sterilized distilled water containing 0.01% Tween 80, literature-based four different concentrations of each microbial treatment were prepared by serial dilutions. Concentrations C1 – C4 corresponded to 1.0×10^5 – 1.0×10^8 conidia/spores mL^{-1} for all entomopathogens except for *S. litura*–NPV for which C1 – C4 corresponded to 1.0×10^3 – 1.0×10^6 OB mL^{-1}. Control treatment was comprised of water containing 0.01% Tween 80. These microbial solutions were applied on potted potato plants using manual atomizer sprayer bottles (50 mL). Fresh potato leaf discs (diameter: 60 mm) were prepared from treated and untreated potted plants and were placed in sterilized Petri-plates pre-lined with 1.0% agar medium. Ten early 3rd instar larvae of *S. litura* were released in each Petri-plate. These Petri-plates were then incubated in an environment chamber (Sanyo MLR-350H, Sanyo, Japan) set at 25 ± 2°C, 70% ± 5 RH and 14:10 hours light: dark photoperiod. Larval mortality was recorded at 2, 4, 8 and 12 days post-exposure. Microbial infection-induced death of larvae was confirmed by shifting them immediately on sterilized plastic Petri plates (diameter: 60 mm) and by examining them daily (Ullah *et al*., 2022).

Table I. Selected botanical insecticides evaluated under laboratory conditions against 3rd instar larvae of *Spodoptera litura*.

Botanical name	Plant species	Formulation	Concentrations used in study (ppm)
Neem oil	*Azadirachta indica*	0.3% EC	120, 60, 30, 15, 7.5
Matrine	*Sophora flavescens*	0.6% EC	180, 90, 45, 22.5, 11.25
Neem extract	*Azadirachta indica*	2.0% SL	480, 240, 120, 60, 30
Nicotine	*Nicotiana tabacum*	10% EC	3040, 1520, 760, 380, 190
Pyrethrin	*Chrysanthemum cinerariaefolium*	5.0% OL	1520,760, 380, 190, 95
Rotenone	*Derris* spp.	7.5% EC	3040, 1520, 760, 380, 190

EC, emulsifiable concentrate; SL, soluble concentrate; OL, oil miscible liquid.

Table II. Selected synthetic insecticides evaluated under laboratory conditions against 3rd instar larvae of *Spodoptera litura*.

Chemical name (active ingredient)	Chemical family*	Mode of action*	Brand name	Company	Label dose (mL ha^{-1})
Chlorantraniliprole	Diamides	Ryanodine receptor modulator	Coragen® 18.5 SC	FMC, Pakistan	100
Chlorfenapyr	Pyrroles	Uncouplers of oxidative phosphorylation	Pirate® 360 SC	Swat Agro Chemicals, Pakistan	200
Fenoxycarb	Fenoxycarb	Juvinile hormone mimic (IGR)	Insegar® 20 SC	Syngenta Pakistan	500
Flubendiamide	Diamides	Ryanodine receptor modulator	Belt 480® SC	Bayer CropScience Pakistan	125
Lufenuron	Benzoylureas	Chitin synthesis inhibitor (IGR)	Match® 50 EC	Syngenta Pakistan	500
Methoxyfenozide	Diacylhydrazines	Ecdysone receptor agonist (IGR)	Runner® 240 SC	Arysta Lifescience Pakistan	500
Spinetoram	Spinosyns	nAChR modulator	Radiant® 120 SC	Arysta Lifescience Pakistan	200

*According to Insecticide Resistance Action Committee (www.irac-online.org) IRAC MoA Classification Version 10.2_23 March 2022. SC = suspension concentrate; EC, emulsifiable concentrate.

Bioassays with synthetic insecticides

Lethal and sublethal effects of selected differential-chemistry non-conventional synthetic insecticides were assessed against 3rd instar larvae of *S. litura* using ventilated plastic Petri-plates (dimensions: 60 × 15 mm). Slightly modified methodology as described by Sharma and Sharma (2018) and Enriquez *et al*. (2010) was followed for these bioassays. Experimental design was completely randomized (CRD) with 10 replications for each treatment. For lethal effects, single concentration based on label-recommended dose of each product was prepared, while half and one-fourth of these label-recommended concentrations were used to determine the sublethal effects of insecticides (Table II). Control treatment included water only. Rest of bioassay procedure including application of insecticidal treatments and exposure of test insects was the same as described above in botanical bioassay. In case of lethal toxicity, larval mortality was recorded at regular time intervals (*i.e.,* at 6, 12, 24, 48 and 72 h) post-exposure, while for sublethal effects, larval development time, pupal weight, pupation time and adult longevity were recorded.

Statistical analysis

Apart from graphical representation, data were statistically analyzed using Statistix® Version 8.1 (Analytical Software, Tallahassee, FL). Prior to analysis, data were corrected using Abbott's formula (Abbott, 1925) and were normalized by arcsine square root transformation. Larval mortality data were subjected to factorial analysis of variance (ANOVA), and the treatment means were further compared using Tukey's highly significant difference (HSD) post-hoc test at 95% level of significance. For analysis of sublethal effects of insecticides, one-way ANOVA was carried out followed by Fisher's least significant difference (LSD) post-hoc test. Median lethal time (LT_{50}) and concentration (LC_{50}) values of all insecticidal treatments were determined by probit analysis (Finney, 1971) using Polo-PC® software (LeOra Software, Parma, MO, USA, 2003).

RESULTS

Comparative toxicity of botanical insecticides against S. litura *larvae*

Bioassay with botanical insecticidal formulations showed significant mortality of *S. litura* larvae by all the treatments at all concentrations (Table III). This mortality response was treatment concentration and exposure time dependent as it increased along with the concentration and time factors. According to factorial analysis of variance, both the treatment ($F_{6, 175}$ = 436.08; $P < 0.001$) and concentration ($F_{4, 175}$ = 227.07; $P < 0.01$) factors, and their interaction ($F_{24, 175}$ = 12.08; $P < 0.001$) had significant effects on the mean mortality of *S. litura* larvae (Table III). Mean maximum mortality was recorded for nicotine (79.33 ± 7.13%), followed by neem oil (77.33 ± 5.84%) and neem extract (69.67 ± 7.50%), while rotenone exhibited minimum mean mortality of *S. litura* larvae (32.33 ± 6.94%) followed by matrine (45.33 ± 7.75%).

Larval repellency bioassay showed significant effect of both the treatments ($F_{5, 342}$ = 49.84; $P < 0.001$) and concentration ($F_{2, 342}$ = 163.40; $P < 0.01$) factors, and their interaction ($F_{10, 342}$ = 3.10; $P < 0.001$) on the larval repellency (Table IV). For all lethal concentrations, neem oil caused maximum repellency followed by neem extract and nicotine, while rotenone and matrine exhibited minimum repellency (Table IV). At LC_{10}, the maximum repellency was exhibited by neem oil (54%) followed by pyrethrin (48%), while matrine caused minimum repellency (18%). At LC_{30}, neem oil and neem extract showed maximum

repellency (64%), whereas rotenone exhibited minimum repellency (30%). Similarly, neem oil, neem extract and nicotine caused significantly maximum larval repellency (98, 92 and 84%, respectively) at their LC_{50} concentrations (Table IV).

Probit regression analysis revealed that neem oil was the most toxic botanical formulation against *S. litura* larvae with minimum LC_{50} value (12.32 ppm), followed by nicotine (24.54 ppm) and neem extract (38.01 ppm). Least effective treatments were pyrethrin and rotenone with maximum LC_{50} values (Table V). According to LT_{50} values, fast acting botanicals against 3rd instar larvae of *S. litura* were nicotine, neem oil and neem extract with LT_{50} values of 11.19 h (7.99–13.98), 16.67 h (13.87–19.28) and 11.68 h (8.58–14.33), respectively (Table VI).

Effectiveness of microbial insecticides against S. litura *larvae*

Microbial insecticides exhibited significant mortality of 3rd instar *S. litura* larvae for all concentrations, and this mortality response was treatment concentration and exposure time dependent. Factorial analysis of variance

Table III. Percent mortality (mean ± S.E.) of 3rd instar larvae of *Spodoptera litura* bioassayed against different botanical insecticides under laboratory conditions.

Botanical concentration	Neem extract ^{B}	Matrine ^{D}	Neem oil ^{A}	Nicotine ^{A}	Pyrethrin ^{C}	Rotenone ^{E}	df	F	P
C1	38.33 ± 5.16 b	20.00 ± 9.83 c	40.00 ± 5.10 ab	51.67 ± 4.96 a	36.67 ± 7.53 b	15.00 ± 5.48 c	5	10.98	< 0.001
C2	53.33 ± 4.94 b	28.33 ± 5.48 d	66.67 ± 7.89 a	66.67 ± 8.16 a	38.33 ± 8.24 c	25.00 ± 7.53 d	5	47.00	< 0.001
C3	73.33 ± 10.33 b	35.00 ± 8.94 d	86.67 ± 6.24 a	86.67 ± 5.27 a	53.33 ± 5.48 c	28.33 ± 5.15 d	5	75.15	< 0.001
C4	86.67 ± 5.08 a	60.00 ± 8.19 b	95.00 ± 3.95 a	95.00 ± 5.16 a	55.00 ± 8.37 bc	46.67 ± 7.92 c	5	55.57	< 0.001
C5	96.67 ± 5.16 a	83.33 ± 7.96 b	98.33 ± 4.08 a	96.67 ± 6.01 a	75.00 ± 8.51 c	56.67 ± 8.11 d	5	52.49	< 0.001

Lowercase letters in the same row indicate significant difference among the treatment means (one-way ANOVA; LSD post-hoc test at α = 0.05), while uppercase letters beside botanical names indicate overall significant difference among the botanical insecticides (factorial ANOVA; HSD post-hoc test at α = 0.05).

Table IV. Percent repellency (mean ± S.E.) of 3rd instar larvae of *Spodoptera litura* exhibited by different lethal concentrations of botanical insecticides under laboratory conditions.

Lethal concentration	Neem extract^{B}	Matrine^{C}	Neem oil^{A}	Nicotine^{B}	Pyrethrin^{B}	Rotenone^{C}	df	F	P
LC_{10}	40.10 ± 5.96bc	18.00 ± 4.67d	54.30 ± 4.28a	42.10 ± 3.59ab	48.00 ± 4.42ab	28.20 ± 4.42cd	5	8.23	< 0.001
LC_{30}	64.00 ± 4.00a	40.10 ± 4.22bc	64.10 ± 4.99a	46.00 ± 4.27b	52.20 ± 3.90ab	30.00 ± 4.47c	5	9.37	< 0.001
LC_{50}	92.20 ± 3.27ab	52.00 ± 5.33d	98.80 ± 2.00a	84.50 ± 4.50b	70.30 ± 5.37c	52.10 ± 4.58d	5	22.11	< 0.001

Lowercase letters in the same row indicate significant difference among the treatment means (one-way ANOVA; LSD post-hoc test at α = 0.05), while uppercase letters beside botanical names indicate overall significant difference among the botanical insecticides (factorial ANOVA; HSD post-hoc test at α = 0.05). LC10, LC30 and LC values of botanical treatments are given in Table I.

Table V. Median lethal concentration (LC_{50}) values for selected botanical insecticidal formulations evaluated against 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatment	LC_{50} (ppm)	Lower and upper 95% fiducial limits (ppm)	X^2 (df = 28)*	P-value	Slope	Intercept
Neem oil	12.32	9.96 – 14.66	127.41	< 0.001	1.686±0.06	1.839±0.09
Matrine	55.53	46.94 – 66.45	127.11	< 0.001	1.471±0.06	2.566±0.10
Neem extract	38.01	30.32 – 45.54	153.39	< 0.001	2.081±0.08	3.291±0.16
Nicotine	24.54	13.98 – 29.22	237.35	< 0.001	1.659±0.07	3.758±0.21
Pyrethrin	333.84	246.83 – 442.41	114.77	< 0.001	0.806±0.05	2.031±0.14
Rotenone	2981.43	2137.60– 4919.58	83.04	< 0.001	0.820±0.06	2.851±0.17

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

Table VI. Median lethal time (LT_{50}) values for selected botanical insecticidal formulations evaluated against 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatment	LT_{50} (hr)	Lower and upper 95% fiducial limits (hr)	X^2 (df = 22)*	P-value	Slope	Intercept
Neem oil	16.67	13.87 – 19.28	99.91	< 0.001	2.269±0.10	2.772±0.15
Matrine	37.59	32.17 – 44.58	148.41	< 0.001	2.208±0.09	3.478±0.15
Neem extract	11.68	8.58 – 14.33	152.77	< 0.001	2.479±0.12	2.647±0.16
Nicotine	11.19	7.99 – 13.98	121.69	< 0.001	2.067±0.11	2.168±0.15
Pyrethrin	26.93	21.82 – 32.45	79.84	< 0.001	1.285±0.09	1.839±0.13
Rotenone	92.28	68.45 – 153.44	52.27	< 0.001	0.964±0.93	1.895±0.14

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

Table VII. Percent mortality (mean ± S.E.) of 3rd instar larvae of *Spodoptera litura* bioassayed against different concentrations of entomopathogenic microbes under laboratory conditions.

Botanical concentration	*Bacillus thuringensis kurstaki* ^{B}	*Beauveria bassiana* ^{D}	*Verticillium lecanii* ^{A}	*Metarhizium anisopliae* ^{A}	*Spodoptera litura–NPV* ^{C}	*Isaria fumosorosea* ^{E}	df	F	P
C1	65.00 ± 4.98 a	43.75 ± 3.78 b	8.75 ± 0.00 d	23.75 ± 3.27 c	71.25 ± 3.13 a	10.00 ± 2.27 d	5	69.04	< 0.001
C2	70.00 ± 5.49 a	53.75 ± 3.27 b	12.50 ± 2.63 d	27.50 ± 2.95 c	78.75 ± 2.67 a	12.50 ± 2.63 d	5	72.27	< 0.001
C3	87.50 ± 2.95 a	57.50 ± 4.41 b	15.00 ± 2.63 d	36.25 ± 3.66 c	85.00 ± 3.24 a	13.75 ± 2.67 d	5	99.05	< 0.001
C4	91.25 ± 2.50 a	67.50 ± 5.15 b	17.50 ± 2.95 d	31.25 ± 3.13 c	96.25 ± 1.64 a	18.75 ± 1.89 d	5	134.33	< 0.001

Concentrations C1 – C4 were 1.0 × 105 – 1.0 × 108 conidia/spores mL-1 for all entomopathogens except for *S. litura* NPV for which C1 – C4 were 1.0 × 103 – 1.0 × 106 OB mL-1. Lowercase letters in the same row indicate significant difference among the treatment means (one-way ANOVA; LSD post-hoc test at α = 0.05), while uppercase letters beside microbe names indicate overall significant difference among the microbial insecticides (factorial ANOVA; HSD post-hoc test at α = 0.05).

Table VIII. Median lethal concentration (LC_{50}) values for promising entomopathogenic microbes evaluated against 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatment	LC_{50} (conidia or spore or OB mL^{-1})	Lower and upper 95% fiducial limits (conidia or spore or OB mL^{-1})	X^2 (df = 30)*	P value	Slope	Intercept
Bacillus thuringensis kurstaki	1.22×10^7	3.52×10^5– 6.34×10^9	249.28	< 0.001	0.351±0.24	0.733±0.11
Beauveria bassiana	5.59×10^6	4.55×10^5 – 2.10×10^7	171.33	< 0.001	0.192±0.02	1.106±0.13
Spodoptera litura–NPV	3.78×10^3	$1.44E \times 10^2$ – 2.04×10^4	153.52	< 0.001	0.349±0.03	0.550±0.11

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

revealed a significant impact of microbial treatments ($F_{5, 168}$= 128.71; P < 0.001), their concentrations ($F_{3, 168}$ = 268.90; P < 0.01), and their interaction ($F_{15, 168}$ = 3.60; P < 0.001) on *S. litura* mortality (Table VII). Highest larval mortality was caused by *S. litura*-NPV (71.25–96.25%) and *B. thuringensis kurstaki* (65.01–91.25%), followed by *B. bassiana* (43.75–67.50%), while minimum larval mortality was exhibited by *V. lecanii* (8.75–17.50%) and *I. fumosorosea* (10.00–18.75%). Mean maximum larval mortality was caused by *S. litura*-NPV (82.81 ± 2.67%), followed by *B. thuringensis kurstaki* (78.44 ± 3.98%) and both these treatments were significantly different from other three microbial insecticides, while *V. lecanii* and *I. fumosorosea* exhibited minimum larval mortality *i.e.* 13.44 ± 2.05% and 13.75 ± 2.36%, respectively (Table VII).

According to probit analysis, *S. litura*-NPV was the most effective microbial insecticide (LC_{50}= 3.78×10^3 OB mL^{-1}), followed by *B. thuringensis kurstaki* (1.22×10^7 spores mL^{-1}) and *B. bassiana* (5.59×10^6 conidia mL^{-1}) (Table VIII), while the most fast-acting microbial insecticide was *B. thuringensis kurstaki* with a LT_{50} value of 3.71 days (3.11–4.31), followed by *S. litura*-NPV (3.83 days), while maximum medial lethal time was recorded for *B. bassiana* (8.88 days) (Table IX).

Lethal and sublethal effects of synthetic insecticides on S. litura *larvae*

Furthermore, some *in-vitro* bioassays were conducted

to assess seven synthetic insecticides having differential-chemistry and modes of action than the conventional ones against 3rd instar larvae of *S. litura*. In first bioassay, data regarding larval mortality by label-recommended dose rates of the insecticides recorded at different time intervals was subjected two-factor factorial analysis of variance which exhibited that treatments ($F_{7, 240} = 289.12$, $P < 0.001$), time ($F_{4, 240} = 630.76$, $P < 0.01$) and their interactions ($F_{28, 240} = 24.99$, $P < 0.001$) had statistically significant effect on *S. litura* mortality (Table X).

All insecticides caused significant larval mortality recorded at each time interval (Table X). The mortality in control ranged from 0.00 to 5.71%. At 6 h post-treatment, flubendiamide gave maximum mortality (10.00 ± 2.18%) followed by spinetoram and chlorantraniliprole (5.71 ± 2.02 and 2.86±1.74%, respectively), while methoxyfenozide and chlorfenapyr revealed no mortality. Similar trend of mortality was observed at 12, 24 and 48 h post-treatment. Trend of mortality changed at 72 h post-exposure where chlorantraniliprole caused maximum and significant mortality (88.57±2.94%) followed by flubendiamide (84.29 ± 2.02%) and spinetoram (77.14 ± 2.86%), while fenoxycarb and methoxyfenozide showed minimum larval mortality (37.14 and 35.71%, respectively) (Table X).

Table IX. Median lethal time (LT_{50}) values for promising entomopathogenic microbes evaluated against 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatment	LT_{50} (days)	Lower and upper 95% fiducial limits (days)	X^2 (df = 30)*	P-value	Slope	Intercept
Bacillus thuringensis kurstaki	3.71	3.11 – 4.31	210.37	< 0.001	2.031±0.08	1.157±0.06
Beauveria bassiana	8.88	7.61 – 10.81	172.97	< 0.001	1.892±0.09	1.791±0.07
Spodoptera litura–NPV	3.83	3.42– 4.25	184.73	< 0.001	2.911±0.09	1.689±0.07

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

Table X. Percent mortality (mean ± S.E.) of 3rd instar larvae of *Spodoptera litura* bioassayed against differential-chemistry synthetic insecticides at their label recommended dose rates.

Time interval (h)	Lufenuron[D]	Spinetoram[B]	Fenoxycarb[F]	Flubendiamide[A]	Chlorantraniliprole[C]	Methoxyfenozide[F]	Chlorfenapyr[E]	df	F	P
6	1.43±1.43bc	5.71±2.02ab	1.43±1.43bc	10.00±2.18a	2.86±1.84bc	0.00±0.00c	0.00±0.00c	5	5.67	0.0002
12	17.14±2.86b	37.14±2.86a	4.29±2.02c	42.86±2.86a	17.14±2.86b	14.29±2.02b	15.71±2.02b	5	29.11	< 0.001
24	31.43±2.61c	50.00±3.09b	18.57±2.61de	60.00±3.09a	28.57±2.61c	14.29±2.02e	24.29±2.02cd	5	40.97	< 0.001
48	44.29±2.02c	64.29±2.02b	22.86±1.84d	74.29±2.02a	70.00±2.18ab	24.29±2.02d	38.57±3.40c	5	88.55	< 0.001
72	60.00±3.09c	77.14±2.86b	37.14±3.60d	84.29±2.02ab	88.57±2.61a	35.71±2.97d	52.86±2.86c	5	56.40	< 0.001

Lowercase letters in the same row indicate significant difference among the treatment means (one-way ANOVA; LSD post-hoc test at α = 0.05), while uppercase letters beside chemical names indicate overall significant difference among the synthetic insecticides (factorial ANOVA; HSD post-hoc test at α = 0.05).

Table XI. Median lethal time (LT_{50}) values for selected non-conventional synthetic insecticides evaluated against 3rd instar larvae of *Spodoptera litura* under laboratory conditions.

Treatment	LT_{50} (h)	Lower and upper 95% fiducial limits (hr)	X^2 (df = 33)*	P-value	Slope	Intercept
Chlorantraniliprole	30.78	27.78 – 34.21	183.192	< 0.001	2.780±0.08	4.138±0.12
Chlorfenapyr	64.30	55.16 – 77.89	115.21	< 0.001	1.745±0.07	3.155±0.11
Fenoxycarb	117.34	88.73 – 178.88	157.91	< 0.001	1.606±0.9	3.323±0.13
Flubendiamide	19.58	17.44 – 21.87	121.35	< 0.001	1.901±0.06	2.454±0.09
Lufenuron	51.26	44.69 – 60.37	125.53	< 0.001	1.788±0.07	3.057±0.11
Methoxyfenozide	139.80	100.82 – 230.51	131.20	< 0.001	1.331±0.08	2.956±0.12
Spinetoram	26.25	23.07– 29.96	149.42	< 0.001	1.785±0.06	2.534±0.09

*Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

Table XII. Effect of sublethal doses of selected differential-chemistry synthetic insecticides on different biological parameters of *Spodoptera litura* under laboratory conditions.

Biological parameters	Lufenuron	Spinetoram	Flubendi-amide	Chlorantra-niliprole	Chlorfenapyr	Control	df	F	P
Half of the label recommended dose rates									
Larval development time (days)	14.21±0.42e	16.64±0.72c	19.51±0.47a	17.77±0.75b	15.25±0.64d	12.47±0.83f	5	150.0	< 0.001
Pupal weight (mg)	218.87±2.74c	211.76±2.03d	201.94±4.46e	221.24±2.86b	225.65±2.12b	241.05±3.40a	5	187.0	< 0.001
Pupal duration (days)	11.49±0.97b	12.55±1.17a	11.75±1.01ab	11.36±1.08b	11.63±1.15ab	7.67±0.80c	5	27.4	< 0.001
Adult longevity (days)	12.20±1.00b	12.27±0.88b	11.83±0.96b	12.21±0.90b	12.06±0.81b	13.80±0.74a	5	6.4	0.001
One-fourth of the label recommended dose rates									
Larval development time (days)	15.96±0.56bc	15.65±0.77c	19.63±0.72a	17.20±0.70b	16.50±0.82cd	14.85±0.75d	5	56.0	< 0.001
Pupal weight (mg)	224.87±2.97d	227.71±5.64cd	228.81±6.18cd	233.98±4.48b	231.06±5.22bc	239.77±1.51a	5	13.0	< 0.001
Pupal duration (days)	9.89±1.03a	9.56±0.87ab	9.88±0.83a	9.32±0.88 ab	9.12±0.12b	7.57±0.63c	5	12.1	< 0.001
Adult longevity (days)	12.77±1.09b	12.51±0.80bc	11.85±0.43c	12.59±0.90b	12.28±0.79bc	13.63±0.79a	5	5.2	0.001

Second instar *S. litura* larvae were exposed to half and one-fourth of the recommended dose rates of different insecticide formulations. Values are means (± S.E.) of 10 independent replications for each treatment. Values within a row having different letters are significantly different from each other (one-way ANOVA followed by LSD post-hoc test at $\alpha = 0.05$).

Overall, the most effective insecticides against *S. litura* were chlorantraniliprole, flubendiamide and spinetoram, while fenoxycarb, methoxyfenozide and chlorfenapyr were least effective (Table X). Similar pattern of lethality was exhibited by median lethal time (LT_{50}) values (Table XI). According to probit regression analysis, flubendiamide, spinetoram and chlorantraniliprole were the most fast-acting insecticides with minimum LT_{50} values *i.e.* 19.58 h (17.44–21.87), 26.25 h (23.07–29.96) and 30.78 h (27.78–34.21), respectively. Maximum LT_{50} values were recorded for fenoxycarb and methoxyfenozide (Table XI).

In second bioassay, effects of sublethal doses of five most effective insecticides were further assessed on different biological characteristics of *S. litura* including larval development time, pupal weight, pupal duration and adult longevity under laboratory conditions. Results revealed a significant effect of sublethal doses of insecticides on all biological parameters of *S. litura* larvae (Table XII). Larval development time was significantly prolonged by both half and one-fourth doses of flubendiamide and chlorantraniliprole (19.51 ± 0.47 and 19.63 ± 0.72 and 17.77 ± 0.75 and 17.20 ± 0.70 days, respectively) in comparison with control (12.47 ± 0.83 and 14.85 ± 0.75 days). The pupal weight was statistically less when flubendiamide was administered at half dose (201.94 ± 4.46 mg) and lufenuron at one-fourth dose (224.87 ± 2.97 mg), while maximum pupal weight was recorded for the control treatment (241.05 ± 3.40 and 239.77 ± 1.51 mg, respectively). In case of pupal duration, statistically longer duration was recorded for spinetoram (at half dose) and lufenuron (at one-fourth dose). Similarly, adult longevity was significantly decreased for all insecticides as compared to control. Particularly, significant suppression of adult lifespan was exhibited by flubendiamide (11.83 and 11.85 days) and chlorfenapyr (12.28 and 12.06 days) at half and one-fourth dose rates, respectively (Table XII).

DISCUSSION

Armyworm infestations on potato crop have become the growing concern of indigenous farmers in Pakistan. *S. litura* is appearing as a difficult to control pest due to high incidence of resistance being manifested by its field populations against the prevailing conventional synthetic insecticides (Saleem *et al*., 2016; Zhang *et al*., 2022). To this end, we assessed under laboratory conditions the comparative effectiveness of 19 promising biorational insecticidal formulations against 3rd instar larvae of *S. litura* because utilization of such reduced-risk insecticides will improve the food quality by minimizing the ecological risks associated with conventional synthetic pesticides.

Among the tested botanical formulations, nicotine (*N. tabacum*) and neem oil and extract (*A. indica*) appeared as the most effective treatments exhibiting maximum cumulative larval mortality (69–79%) in 72 h exposure, concomitantly with the minimum LC_{50} and LT_{20} values. Similarly, these botanicals showed maximum larval

repellency by their LC_{10}, LC_{30} and LC_{50} values. These results are consistent with the findings of some recent studies demonstrating significant toxicity of *N. tabacum* and *A. indica* extracts against different fall armyworm *S. frugiperda* (Duarte *et al*., 2019; Phambala *et al*., 2020; Hernandez-Trejo *et al*., 2021). Sisay *et al*. (2019) and Phambala *et al*. (2020) revealed significantly higher mortality (50–66%) of 3rd instar larvae of *S. frugiperda* by *N. tabacum* extracts. Apart from different *Spodoptera* species, less larval morality showed by these studies than our results would also be due to the plant extract nature because we used commercial formulations (emulsifiable concentrates) of these plants while above mentioned studies used either aqueous or crude plant extracts. Phyto-constituents derived from *A. indica* have been effectively used since decades against various lepidopterous, coleopterous, dipterous and hemiptrous pests (Isman, 2006; Benelli *et al*., 2017). Nature has blessed this plant with a wide array of alkaloids, phenolics and terpenoids particularly triterpenoids (nimbin, salannin etc.) and other azadirachtin analogues thereof (Isman, 2006). *A. indica* extractives have multifaceted modes of action exhibiting contact and stomach toxicity, ovipositional deterrence, ovicidal, growth inhibitory and antifeedant effects against various insect pests (Chaudhary *et al*., 2017; Isman, 2020). Regarding repellency, our results are also in line with those of Nelson and Venugopal (2006) and Phambala *et al*. (2020) showing maximum feeding deterrence by the extracts of *A. indica* and *N. indica.*

Regarding evaluation of microbial formulations, *S. litura*-NPV and *Bt* kurstaki were the most effective treatments exhibiting 65–96% cumulative larval mortality in 12-days bioassay. These findings corroborate some previous studies which have shown the individual and combined synergistic toxicity of *Spodoptera*-specific NPV strains and *B. thuringiensis* against the larvae *S. frugiperda* under laboratory conditions (Nagal and Verma, 2015; Guido-Cira *et al*., 2017). NPVs are usually highly pathogenic and effective against different lepidopterous pests including many *Spodoptera*, *Helicoverpa* and *Heliothis* species (Ravishankar and Venkatesha, 2010; Beas-Catena *et al*., 2014; Arrizubieta *et al*., 2022). Nagal and Verma (2015) and Suarez-Lopez *et al*. (2022) showed significant pathogenicity of *S. litura*-NPV against 3rd instar larvae of *S. litura* and *S. littoralis* with LC_{50} values of 1.32×10^5 and 6.6×10^5 OB/ml, respectively. Although *B. bassiana* exhibited considerable (up to 68%) larval mortality, other two entomopathogenic fungal formulations (*M. anisopliae* and *I. fumosorosea*) tested in this study did not show significant toxicity against *S. litura*. Our results are in contrast to Batool *et al*. (2022) and Ullah *et al*. (2022) demonstrating the indigenous strains of *M. anisopliae* as the most effective EPF against 3rd instar larvae of *S. litura* and *S. frugiperda*, respectively. This is possibly due to the fact that different biogeographic strains of EPF can vary in their pathogenicity and virulence against target insect pests because of their genetic diversity and differential molecular and enzymatic characteristics (Maistrou *et al*., 2020).

In third bioassay with non-conventional synthetic insecticides, the most toxic and fast-acting insecticides were flubendiamide, chlorantraniliprole and spinetoram with significantly maximum larval mortality and minimum LT_{50} values. Similarly, significant suppression of life-table parameters was exhibited by sublethal doses of flubendiamide and chlorantraniliprole. Flubendiamide is novel diamide group insecticide and is highly effective against lepidopterous larvae including *S. litura* (Tohnishi *et al*., 2005; Maqsood *et al*., 2018). Our results affirm the findings of Nagal and Verma (2015) and Thakur and Srivastava (2019) that diamides (chlorantraniliprole and flubendiamide) and spinosyns (spinetoram and spinosad) are effective differential-chemistry reduced-risk insecticides against 3rd instar larvae of *S. litura*. Hannig *et al*. (2009), Liu *et al*. (2017) and Kong *et al*. (2021) demonstrated chlorantraniliprole as an effective biorational alternate to conventional synthetic insecticides exerting lethal and sublethal effects on moths and larvae of *S. litura*, *S. exigua*, *Agrotis ipsilon* and *Helicoverpa armigera*. Likewise, significant toxicity of chlorantraniliprole, either alone or in combination with an indigenous isolate of *M. anisopliae*, has been shown against *S. litura* 3rd instar larvae by Batool *et al*. (2022).

Nevertheless, it would be imperative to look for the compatibility of these effective botanical (*N. tabacum* and *A. indica* extracts), microbial (NPV, *B. thuringiensis* and *B. bassiana*) and differential chemistry synthetic (flubendiamide, chlorantraniliprole and spinetoram) insecticidal treatments among themselves with other biorational or reduced-risk pesticides. For instance, some recent studies have demonstrated the synergistic action of *S. litura*-NPV with emamectin benzoate, lufenuron and spinosad (Yasin *et al*., 2020; Suarez-Lopez *et al*., 2022; Ayyub *et al*., 2019; Dáder *et al*., 2020; Thakur *et al*., 2022). Similarly, *B. thuringiensis* have shown synergistic action against 3rd instar larvae of *S. littoralis* when applied in combination with spinosad and cypermethrin (El-Sheikh 2012; Abd El-Samei *et al*., 2019) and against 3rd instar larvae of Indian meal moth (*Plodia interpunctella*) (Nouri-Ganbalani *et al*., 2016).

CONCLUSIONS

In brief, this laboratory study revealed the

effectiveness of aforementioned botanical, microbial and non-conventional synthetic insecticides, particularly of *A. indica*, *N. tabacum*, *B. thuringensis kurstaki*, *S. litura*-NPV, flubendiamide and spinetoram, against 3rd instar larvae of *S. litura*. However, further *in-vitro* and *in-situ* assessment of the combinations of these effective treatments and their lethal and sublethal impacts on insect natural enemies and on other non-target species constitute the future perspectives of this work.

ACKNOWLEDGMENTS

Authors acknowledge the technical help and valuable advice given by Muhammad Asam Riaz during the preparation and proofreading of the draft.

Funding

This study was partly funded by NRPU Research Project (No. 6702) of the Higher Education Commission of Pakistan.

Ethical statement

Authors declare that this study did not require ethical committee's approval or any other ethical considerations.

Statement of conflicts of interest

The authors have declared no conflict of interest.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.*, **18**: 265–267. https://doi.org/10.1093/jee/18.2.265a

Abd El-Samei, E.M., Hamama, H.M., El-Enien, M.G.A.A. and Awad, H.H., 2019. Interaction of spinosad and *Bacillus thuringiensis* on certain toxicological, biochemical and molecular aspects in the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Afr. Entomol.*, **27**: 508–522. https://doi.org/10.4001/003.027.0508

Abdullah, A., Ullah, M.I., Raza, A.B.M., Arshad, M and Afzal, M., 2019. Host plant selection affects biological parameters in armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Pakistan J. Zool.*, **51**: 2117–2123. https://doi.org/10.17582/journal.pjz/2019.51.6.2117.2123

Acheuk, F., Basiouni, S., Shehata, A.A., Dick, K., Hajri, H., Lasram, S. and Ntougias, S., 2022. Status and prospects of botanical biopesticides in Europe and mediterranean countries. *Biomolecules*, **12**: 311. https://doi.org/10.3390/biom12020311

Ahmad, M., and Gull, S., 2017. Susceptibility of armyworm *Spodoptera litura* (Lepidoptera: Noctuidae) to novel insecticides in Pakistan. *Can. Entomol.,* **149**: 649–661. https://doi.org/10.4039/tce.2017.29

Ahmad, M., Ghaffar, A., Rafiq, M. and Ali, P.M., 2013. Host plants of leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. *Asian J. Agric. Biol.,* **1**: 23–28.

Ahmed, K.S., Idrees, A., Majeed, M.Z., Majeed, M.I., Shehzad, M.Z., Ullah, M.I., Afzal, A. and Li, J., 2022. Synergized toxicity of promising plant extracts and synthetic chemicals against fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in Pakistan. *Agronomy,* **12**: 1289. https://doi.org/10.3390/agronomy12061289

Arrizubieta, M., Simón, O., Ricarte-Bermejo, A., López-Ferber, M., Williams, T. and Caballero, P., 2022. Coocclusion of *Helicoverpa armigera* single nucleopolyhedrovirus (HearSNPV) and *Helicoverpa armigera* multiple nucleopolyhedrovirus (HearMNPV): Pathogenicity and stability in homologous and heterologous hosts. *Viruses*, **14:** 687. https://doi.org/10.3390/v14040687

Arthurs, S. and Dara, S.K., 2019. Microbial biopesticides for invertebrate pests and their markets in the United States. *J. Invertebr. Pathol.*, **165**: 13–21. https://doi.org/10.1016/j.jip.2018.01.008

Ayyub, M.B. Nawaz, A., Arif, M.J. and Amrao, L., 2019. Individual and combined impact of nuclear polyhedrosis virus and spinosad to control the tropical armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), in cotton in Pakistan. *Egypt. J. Biol. Pest Contr.*, **29**: 1–6. https://doi.org/10.1186/s41938-019-0170-4

Batool, Z., Riaz, M.A., Sayed, S., Majeed, M.Z., Ahmed, S. and Ullah, S., 2022. *In vitro* synergy of entomopathogenic fungi and differential-chemistry insecticides against armyworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Int. J. Trop. Insect Sci.*, **42**: 1997–2006. https://doi.org/10.1007/s42690-022-00751-4

Beas-Catena, A., Sánchez-Mirón, A., García-Camacho, F., Contreras-Gómez, A. and Molina-Grima, E., 2014. Baculovirus biopesticides: An overview. *J. Anim. Pl. Sci.,* **24**: 362–373.

Benelli, G., Canale, A., Toniolo, C., Higuchi, A., Murugan, K., Pavela, R. and Nicoletti, M., 2017. Neem (*Azadirachta indica*): Towards the ideal insecticide? *Nat. Prod. Res.*, **31**: 369–386. https://doi.org/10.1080/14786419.2016.1214834

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.A., Miret, J.A.J., Justesen, A.F., Magnusson, C.S., Milonas, P. and Navas-Cortes, J.A., 2019. Pest categorisation of *Spodoptera litura*. *EFSA J.*, **17**: 5765. https://doi.org/10.2903/j.efsa.2019.5765

Chaudhary, S., Kanwar, R.K., Sehgal, A., Cahill, D.M., Barrow, C.J., Sehgal, R. and Kanwar, J.R., 2017. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Front. Pl. Sci.*, **8**: 610. https://doi.org/10.3389/fpls.2017.00610

Copping, L.G., and Menn, J.J., 2000. Biopesticides: A review of their action, applications and efficacy. Pest management science. *J. Pestic. Sci.*, **56**: 651–676. https://doi.org/10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U

Dáder, B., Aguirre, E., Caballero, P. and Medina, P., 2020. Synergy of lepidopteran nucleopolyhedroviruses AcMNPV and SpliNPV with insecticides. *Insects*, **11**: 316. https://doi.org/10.3390/insects11050316

Duarte, J.P., Redaelli, L.R., Jahnke, S.M. and Trapp, S., 2019. Effect of *Azadirachta indica* (Sapindales: Meliaceae) oil on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae and adults. *Fla. Entomol.*, **102**: 408–412. https://doi.org/10.1653/024.102.0218

Duso, C., Pozzebon, A., Lorenzon, M., Fornasiero, D., Tirello, P., Simoni, S. and Bagnoli, B., 2022. The impact of microbial and botanical insecticides on grape berry moths and their effects on secondary pests and beneficials. *J. Agron.*, **12**: 217. https://doi.org/10.3390/agronomy12010217

El-Sheikh, T.A.A., 2012. Biological, biochemical and histological effects of spinosad, *Bacillus thuringiensis* var. *kurstaki* and cypermethrin on the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Egypt. Acad. J. biol. Sci.*, **4**: 113–124. https://doi.org/10.21608/eajbsc.2012.16130

Enriquez, C.R., Pineda, S., Figueroa, J.I., Schneider, M. and Martínez, A., 2010. Toxicity and sublethal effects of Methoxyfenozide on *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. econ. Ent.*, **103**: 662–667. https://doi.org/10.1603/EC09244

Finney, D.J., 1971. Statistical logic in the monitoring of reactions to therapeutic drugs. *Method. Inform. Med.*, **10**: 237–245. https://doi.org/10.1055/s-0038-1636052

Granados-Echegoyen, C., Loera-Alvarado, G., Miranda-Salcedo, M.A., Hernández-Cruz, J., Luna-Cruz, A. and Loera-Alvarado, E., 2021. Field efficacy of synthetic and botanical-derived insecticides against *Melanaphis sacchari*, and non-target and beneficial species associated with cultivated sorghum. *Southw. Entomol.*, **46**: 33-46. https://doi.org/10.3958/059.046.0103

Guido-Cira, N.D., Tamez-Guerra, P., Mireles-Martínez, M., Villegas-Mendoza, J.M. and Rosas-García, N.M., 2017. Activity of *Bacillus thuringiensis* and baculovirus based formulations to *Spodoptera* 1 Species. *Southw. Entomol.*, **42**: 191–201. https://doi.org/10.3958/059.042.0118

Hannig, G.T., Ziegler, M. and Marcon, P.G., 2009. Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode of action groups. *Pest Manage. Sci.*, **65**: 969–974. https://doi.org/10.1002/ps.1781

Hernandez-Trejo, A., Rodríguez-Herrera, R., Sáenz-Galindo, A., López-Badillo, C.M., Flores-Gallegos, A.C., Ascacio-Valdez, J.A. and Osorio-Hernández, E., 2021. Insecticidal capacity of polyphenolic seed compounds from neem (*Azadirachta indica*) on *Spodoptera frugiperda* (JE Smith) larvae. *J. Environ. Sci. Hlth.*, **Part B**: 1–8. https://doi.org/10.1080/03601234.2021.2004853

Idrees, A., Qadir, Z.A., Afzal, A., Ranran, Q. and Li, J., 2022. Laboratory efficacy of selected synthetic insecticides against second instar invasive fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *PLoS One*, **17**: e0265265. https://doi.org/10.1371/journal.pone.0265265

Ishaaya, I. and Degheele, D., 1998. *Insecticides with novel modes of action*: *Mechanisms and application*. Springer-Verlag, Berlin. Heidelberg, Germany. https://doi.org/10.1007/978-3-662-03565-8

Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annls Rev. ent.*, **51**: 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146

Isman, M.B., 2015. A renaissance for botanical insecticides? *Pest Manage. Sci.*, **71**: 1587–1590. https://doi.org/10.1002/ps.4088

Isman, M.B., 2020. Botanical insecticides in the twenty-first century fulfilling their promise?. *Annls Rev. Ent.*, **65**: 233–249. https://doi.org/10.1146/annurev-ento-011019-025010

Jin, T., Lin, Y.Y., Chi, H., Xiang, K.P., Ma, G.C., Peng, Z.Q. and Yi, K.X., 2020. Comparative performance of the fall armyworm (Lepidoptera: Noctuidae) reared on various cereal-based artificial diets. *J. econ. Ent.*, **113**: 2986–2996. https://doi.org/10.1093/jee/toaa198

Khan, M.A. and Ahmad, W., 2019. Synthetic chemical insecticides: Environmental and agro contaminants. In: *Microbes for sustainable insect pest management. Sustainability in plant and crop protection* (eds. M. Khan and W. Ahmad). Springer, pp. 1–22. https://doi.org/10.1007/978-3-030-23045-6_1

Kong, F., Song, Y., Zhang, Q., Wang, Z., and Liu, Y., 2021. Sublethal effects of chlorantraniliprole on *Spodoptera litura* (Lepidoptera: Noctuidae) moth: Implication for attract-and-kill strategy. *Toxics*, **9**: 20. https://doi.org/10.3390/toxics9020020

Lacey, L.A., 2017. Entomopathogens used as microbial control agents. In: *Microbial control of insect and mite pests.* Academic Press, pp. 3–12. https://doi.org/10.1016/B978-0-12-803527-6.00001-9

Landero-Valenzuela, N., Alonso-Hernández, N., Lara-Viveros, F., Gómez-Domínguez, N.S., Juárez-Pelcastre, J., Agua-do-Rodríguez, J., Luna-Cruz, A., Lagunez-Rivera, L., Aguilar-Pérez, L.A., Hinojosa-Garro, D. and Granados-Echegoyen, C., 2022. Efficiency of *Schinus molle* essential oil against *Bactericera cockerelli* (Hemiptera: Triozidae) and *Sitophilus zeamais* (Coleoptera: Dryophthoridae). *Agriculture*, **12**: 554. https://doi.org/10.3390/agriculture12040554

Liu, Y., Gao, Y., Liang, G. and Lu, Y., 2017. Chlorantraniliprole as a candidate pesticide used in combination with the attracticides for lepidopteran moths. *PLoS One*, **12**: e0180255. https://doi.org/10.1371/journal.pone.0180255

Lo Pinto, M., Vella, L., and Agrò, A., 2022. Oviposition deterrence and repellent activities of selected essential oils against Tuta absoluta Meyrick (Lepidoptera: Gelechiidae): laboratory and greenhouse investigations. *Int. J. Trop. Insect Sci.,* **42**: 3455-3464. https://doi.org/10.1007/s42690-022-00867-7

Maistrou, S., Natsopoulou, M.E., Jensen, A.B. and Meyling, N.V., 2020. Virulence traits within a community of the fungal entomopathogen *Beauveria*: Associations with abundance and distribution. *Fungal Ecol.*, **48**: 100992. https://doi.org/10.1016/j.funeco.2020.100992

Mansour, R., and Biondi, A., 2021. Releasing natural enemies and applying microbial and botanical pesticides for managing *Tuta absoluta* in the MENA region. *Phytoparasitica,* **49**: 179–194. https://doi.org/10.1007/s12600-020-00849-w

Maqsood, S., Afzal, M., Aqueel, M.A., Wakil, W. and Khan, H.A.A., 2018. Comparative evaluation of selected biorational insecticides against *Spodoptera litura* (Fabricius) on cauliflower. *Pakistan J. Zool.,* **50**: 1645-1652. https://doi.org/10.17582/journal.pjz/2018.50.5.1645.1652

Nagal, G. and Verma, K.S., 2015. Intrinsic toxicity evaluation of novel insecticides and bio-pesticides against *Spodoptera litura* (Fabricius) on Bell pepper. *Annls Pl. Prot. Sci.*, **23**: 227–230.

Narciso, J., Ormskirk, M., Jones, S., Rolston, P., Moran-Diez, M.E., Hurst, M. and Glare, T., 2021. Using multiple insecticidal microbial agents against diamondback moth larvae-does it increase toxicity? *N. Z. J. agric. Res.*, **64**: 178–193. https://doi.org/10.1080/00288233.2019.1582074

Nathan, S.S. and Kalaivani, K., 2006. Combined effects of *Azadirachtin* and nucleopolyhedrovirus (SpltNPV) on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) larvae. *Biol. Contr.*, **39**: 96–104. https://doi.org/10.1016/j.biocontrol.2006.06.013

Nelson, S.J. and Venugopal, M.S., 2006. Antifeedant and growth disruptive effects of various plant products on *Spodoptera litura* F. (Lepidoptera: Noctuidae). *J. entomol. Res*., **30**: 93–102.

Nouri-Ganbalani, G., Borzoui, E., Abdolmaleki, A., Abedi, Z. and George-Kamita, S., 2016. Individual and combined effects of *Bacillus thuringiensis* and azadirachtin on *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *J. Insect Sci.*, **16**: 1–8. https://doi.org/10.1093/jisesa/iew086

Oerke, E.C., Dehne, H.W., Schonbeck, F. and Weber, A., 1994. *Crop production and crop protection estimated losses in major food and cash crops*. Elsevier Science, Amsterdam:, pp. 808.

Paul, D. and Chaudhary, M., 2016. Larvicidal and antifeedant activity of some indigenous plants of Meghalaya against 4th instar *Helicoverpa armigera* (Hübner) larvae. *J. Crop. Prot.*, **5**: 447–460. https://doi.org/10.18869/modares.jcp.5.3.447

Phambala, K, Tembo, Y., Kasambala, T., Kabambe, V.H., Stevenson, P.C. and Belmain, S.R., 2020. Bioactivity of common pesticidal plants on fall armyworm larvae (*Spodoptera frugiperda*). *Plants*, **9**: 112. https://doi.org/10.3390/plants9010112

Qadir, Z.A., Idrees, A., Mahmood, R., Sarwar, G., Bakar, M.A., Ahmad, S., Raza, M.M. and Li, J., 2021. Effectiveness of different soft acaricides against honey bee ectoparasitic mite *Varroa destructor* (Acari: Varroidae). *Insects*, **12**: 1032. https://doi.org/10.3390/insects12111032

Rai, D., Updhyay, V., Mehra, P., Rana, M. and Pandey, A.K., 2014. Potential of entomopathogenic fungi as biopesticides. *Ind. J. Sci. Res. Tech.*, **2**: 7–13. https://doi.org/10.5958/2230-732X.2014.01380.1

Rani, A.T., Kammar, V., Keerthi, M.C., Rani, V., Majumder, S., Pandey, K.K., Singh, J., 2021. Biopesticides: An alternative to synthetic insecticides. In: *Microbial technology for sustainable environment.* Springer, Singapore, pp. 439–466. https://doi.org/10.1007/978-981-16-3840-4_23

Ravishankar, B.S. and Venkatesha, M.G., 2010. Effectiveness of SlNPV of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) on different host plants. *J. Biopestic.*, **3**: 168–171.

Saleem, M., Hussain, D., Ghouse, G., Abbas, M. and Fisher, S.W., 2016. Monitoring of insecticide resistance in *Spodoptera litura* (Lepidoptera: Noctuidae) from four districts of Punjab, Pakistan to conventional and new chemistry insecticides. *J. Crop Prot.*, **79**: 177–184. https://doi.org/10.1016/j.cropro.2015.08.024

Serrão, J.E., Plata-Rueda, A., Martínez, L.C. and Zanuncio, J.C., 2022. Side-effects of pesticides on non-target insects in agriculture: A mini-review. *Sci. Nat.*, **109**: 1–11. https://doi.org/10.1007/s00114-022-01788-8

Shad, S.A., Sayyed, A.H., Fazal, S., Saleem, M.A., Zaka, S.M. and Ali, M., 2012. Field evolved resistance to carbamates, organophosphates, pyrethroids and new chemistry insecticides in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). *J. Pest Sci.*, **85**: 153–162. https://doi.org/10.1007/s10340-011-0404-z

Sharma, S. and Sharma, P.C., 2018. Relative toxicity of novel insecticides against *Spodoptera litura* (Fabricius) field populations. *J. entomol. Res.*, **42**: 41-44. https://doi.org/10.5958/0974-4576.2018.00007.5

Sisay, B., Tefera, T., Wakgari, M., Ayalew, G. and Mendesil, E., 2019. The efficacy of selected synthetic insecticides and botanicals against fall armyworm, *Spodoptera frugiperda*, in Maize. *Insects*, **10**: 45. https://doi.org/10.3390/insects10020045

Sparks, T.C., Crossthwaite, A.J., Nauen, R., Banba, S., Cordova, D., Earley, F., and Wessels, F.J., 2020. Insecticides, biologics and nematicides: Updates to IRAC's mode of action classification a tool for resistance management. *Pestic. Biochem. Physiol.*, **167**: 104587. https://doi.org/10.1016/j.pestbp.2020.104587

Suarez-Lopez, Y.A., Aldebis, H.K., Hatem, A.E.S. and Vargas-Osuna, E., 2022. Interactions of entomopathogens with insect growth regulators for the control of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Biol. Contr.*, **170**: 104910. https://doi.org/10.1016/j.biocontrol.2022.104910

Thakur, H. and Srivastava, R.P., 2019. Sub-lethal and antifeedant effect of spinosyn and diamide insecticides against *Spodoptera litura* (Fab.) and *Spilarctia obliqua* (Wlk.). *J. entomol. Res.*, **43**: 431–438. https://doi.org/10.5958/0974-4576.2019.00076.8

Thakur, N., Tomar, P., Sharma, S., Kaur, S., Sharma, S., Yadav, A.N. and Hesham, A.E.L., 2022. Synergistic effect of entomopathogens against *Spodoptera litura* (Fabricius) under laboratory and greenhouse conditions. *Egypt. J. Biol. Pest Contr.*, **32**: 1–10. https://doi.org/10.1186/s41938-022-00537-3

Tohnishi, M., Nakao, H., Furuya, T., Seo, A., Kodama, H., Tsubata, K. and Nishimatsu, T., 2005. Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. *J. Pestic. Sci.*, **30**: 354–360. https://doi.org/10.1584/jpestics.30.354

Tong, H., Qi, S.u., Xiaomao, Zhou. and Lianyang, Bai., 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *J. Pest Sci.*, **86**: 599–609. https://doi.org/10.1007/s10340-013-0505-y

Ullah, S., Raza, A.B.M., Alkafafy, M., Sayed, S., Hamid, M.I., Majeed, M.Z. and Asim, M., 2022. Isolation, identification and virulence of indigenous entomopathogenic fungal strains against the peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *Egypt. J. Biol. Pest Contr.*, **32**: 1–11. https://doi.org/10.1186/s41938-021-00500-8

Yasin, M., Qazi, M.S., Wakil, W. and Qayyum, M.A., 2020. Evaluation of nuclear polyhedrosis Virus (NPV) and emamectin benzoate against *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). *Egypt. J. Biol. Pest Contr.*, **30**: 1–6. https://doi.org/10.1186/s41938-020-00271-8

Zhang, Z., Gao, B., Qu, C., Gong, J., Li, W., Luo, C. and Wang, R., 2022. Resistance monitoring for six insecticides in vegetable field-collected populations of *Spodoptera litura* from China. *Horticulturae*, **8**: 255. https://doi.org/10.3390/horticulturae8030255