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Synergized Toxicity of Promising Plant Extracts and Synthetic Chemicals against Fall Armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in Pakistan

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Abstract: Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), is a destructive pest of a wide array of agricultural and horticultural crops worldwide. This in vitro research assessed the combined effect of methanolic extracts of indigenous flora of Soone Valley (Khushab, Pakistan) and nine commonly used synthetic insecticides against 3rd instar larvae of *S. frugiperda* using the leaf-dip bioassay method. Toxicity bioassays with twelve plant extracts revealed that the extracts of *Withania somnifera* (L.) Dunal, *Sophora mollis* (Royle) Baker and *Rhazya stricta* Decne. were the most effective, exhibiting minimum LC_{50} and LT_{50} values. Bioassays with synthetic insecticides revealed a significantly higher mortality of *S. frugiperda* larvae by emamectin benzoate (45%), chlorpyrifos (40%) and chlorantraniliprole (38%). Further bioassays with 10 binary combinations of these most effective botanical and synthetic insecticides showed that seven pesticidal combinations exhibited synergistic toxicity, and three combinations comprising emamectin benzoate exhibited an additive effect on the mortality of *S. frugiperda* larvae. GC–MS analyses of methanolic extracts of *W. somnifera*, *S. mollis* and *R. stricta* revealed 1,2,4-trimethyl-benzene and 3,5-dimethyl-octane, 1-ethyl-2-methyl-benzene, and 1-monolinoleoylglycerol trimethylsilyl ether, decane, and lupeol as major bioconstituents, respectively. Our results demonstrated that combining botanicals with synthetic insecticides can synergize their toxicity against *S. frugiperda* larvae, suggesting their potential incorporation into future IPM programs against *S. frugiperda* and other lepidopterous pests.

Keywords: fall armyworm; *Spodoptera frugiperda*; plant extracts; chemicals; synergistic toxicity; binary combinations

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1. Introduction

The fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) is a polyphagous pest of many agricultural and horticultural crops. It is native to the western tropical hemisphere and was recognized as a severe threat to farmers in West and Sub-Saharan Africa in 2016 [1,2]. Later, it was reported that these armyworms were infesting maize crops in China and India in May 2018 [3,4]. In March 2019, this exotic species was reported from various localities in Sindh Province, Pakistan, where they were damaging maize crops [5]. *Spodoptera frugiperda* is a polyphagous pest that infests a wide array of host plants, comprising approximately of 350 plant species from 76 families, including maize,

sorghum, millet, wheat, sugarcane and vegetable crops. Maize and cabbage are the most vulnerable crops to *S. frugiperda* infestation worldwide [6]. Loss of these crops causes an economic loss of approximately 9.4 billion US dollars annually in Africa alone [7].

Synthetic insecticides have been prime and inevitable control options for combating *S. frugiperda* infestations worldwide. In Pakistan, growers rely exclusively upon synthetic pesticides to control lepidopterous pests, including *Spodoptera* species [8,9]. Unfortunately, farmers have reported that the available pesticides do not effectively control *S. frugiperda* in the field. As a result, they arbitrarily increase the labeled dose to eradicate this insect pest, which will lead to the development of insecticide resistance in *S. frugiperda* in the future. The overuse of synthetic insecticides to eradicate this pest is manifested as environmental contamination and insecticidal resistance in *S. frugiperda*. Approximately 46 and 60% of farmers in Ethiopia and Kenya, respectively, claimed the ineffectiveness of synthetic insecticides against *S. frugiperda* [10]. Indeed, repeated applications of insecticides with the same mode of action have resulted in resistance to *S. frugiperda* in Africa [2]. Furthermore, Zhang et al. [11] monitored the resistance in *S. frugiperda* against commonly used insecticides and revealed the evolution of resistance in *S. frugiperda* against chlorpyrifos, spinosad, lambda-cyhalothrin, malathion, fenvalerate, deltamethrin, emamectin benzoate and chlorantraniliprole. Therefore, there is a need to develop an integrated management approach to effectively control this invasive pest.

In response to the global spread of this pest, especially in Pakistan, many studies have recently focused on developing biopesticides with the integration of various control strategies as a component of integrated pest management (IPM) against *S. frugiperda* [12,13]. However, plant-based insecticides have long been recognized as promising alternatives to synthetic insecticides for insect pest management [14,15]. Botanical pesticides are usually environmentally friendly, cost-effective and exhibit relatively low toxicity to on-target organisms [16,17]. Many native plants having the ethnomedicinal value of certain biogeographic regions may also exhibit insecticidal potential. For instance, Soone Valley and its surrounding salt range (Khushab, Punjab, Pakistan) are enriched with flora of ethnomedicinal value and insecticidal potential [18–20]. The comparative toxicity of the extracts of forty plant species, including herbs, shrubs and trees, from this area determined against *Spodoptera litura* by Majeed et al. [21] provides a basis for further research on evaluating the combined insecticidal effect of promising botanicals along with the synthetic insecticide against *S. frugiperda*. Many previous studies have demonstrated the potential of plant-derived compounds to enhance the toxicity and to reduce the inhibitory concentration of different synthetic insecticides [22]. Therefore, this laboratory research aimed to assess the combined toxicity of promising local plant extracts and commonly used synthetic insecticides against the 3rd instar larvae of *S. frugiperda*. In brief, binary and/or tertiary combinations of LC_{33} and LC_{50} of the selected botanical extracts were bioassayed along with half of the label-recommended dose rates of selected synthetic insecticides.

2. Materials and Methods

2.1. Insect Culture

For the rearing of S. frugiperda, mature larvae were collected from the maize field (32°13′35″ N, 72°68′67″ E) and were brought to the laboratory of Entomology, College of Agriculture, University of Sargodha (Punjab, Pakistan). These larvae were reared in glass Petri plates (diameter 9 cm) lined with a corn-based artificial diet [23] under controlled conditions of 25 ± 2 °C, 60 ± 5% RH and 16 h:8 h (L:D) photoperiod. Only few larvae (5–8 larvae per Petri plate) were maintained in order to avoid cannibalism. The larval diet was changed regularly until pupation. Pupae were maintained on moist Whatman No. 1 filter paper (diameter 9 cm) in glass Petri plates. After emergence, adult moths were provided a 10% honey solution and were housed separately in rearing plastic cages (30 × 30 × 30 cm; Bugdorm-I, Taiwan) with hanging muslin cloth strips for oviposition. The egg masses of S. frugiperda were collected from the cages, maintained on Petri plates lined with a thin layer of artificial diet and reared to obtain subsequent generations. Healthy

and active 3rd instar larvae of the laboratory-reared F_3 generation of S. frugiperda were utilized in all bioassays.

2.2. Collection and Extraction of Plant Materials

Samples of promising local plant species, as detailed in Table 1, were collected from six distinct locations (Figure 1) of the Soon Valley and adjacent salt range of district Khushab (Punjab, Pakistan) (Table 2). Collected plants were identified up to the species level with the help of an online identification portal (http://www.theplantlist.org/1.1, accessed on 20 April 2022) generated by the botanical community in response to the Global Strategy for Plant Conservation (GSPC) and by the local experts of the Department of Botany, University of Sargodha, Pakistan. These plant samples were prepared and extracted by a Soxhlet apparatus (DH. WHM-12393, Daihan Scientific, Seoul, Korea) using methanol as the extraction solvent in a 1:10 ratio as described previously [21]. The extraction time for most of the samples was 4–6 h. Further purification of extracted plant samples was performed using a rotary evaporator (WEV-1001 L, Daihan Scientific, South Korea) fitted with a vacuum pump and chiller. Extracted plant materials were stored at 4 °C in 50 mL hermetic dark glass vials until their use in the toxicity bioassays.

Table 1. Description of plant samples collected from the selected sites of Soone Valley (Khushab) and the surrounding salt range of Pakistan.

Plant Species	Vernacular Name	Family	Locality	Part(s) Used
Buxus papillosa C. K. Schneid.	Shamshad	Buxaceae	Kufri	Leaves
Maerua arenaria Hook. f. & Thomson	Hemkand	Capparaceae	Uchhali	leaves
Monotheca buxifolia Falc. A. DC.	Kohair	Sapotaceae	Khura	Leaves
Olea ferruginea Wall. ex Aitch.	Kao	Oleaceae	Uchhali	Leaves
Peganum harmala L.	Harmal	Nitrariaceae	Anga	Leaves
Periploca aphylla Decne.	Jangli bata	Apocynaceae	Anga	Leaves
Rhazya stricta Decne.	Akri	Apocynaceae	Uchhali	Leaves and flowers
Salvia moorcroftiana Wall. ex Benth.	Khalatra	Lamiaceae	Kufri	Leaves
Solanum incanum L.	Mahori	Solanaceae	Kufri	Fruits
Solanum nigrum L.	Black nightshade	Solanaceae	Khabeki	Leaves and flowers
Sophora mollis (Royle) Baker	Kohni	Leguminosae	Khabeki	Leaves and flowers
Withania somnifera (L.) Dunal	Aksan	Solanaceae	Khura	Roots

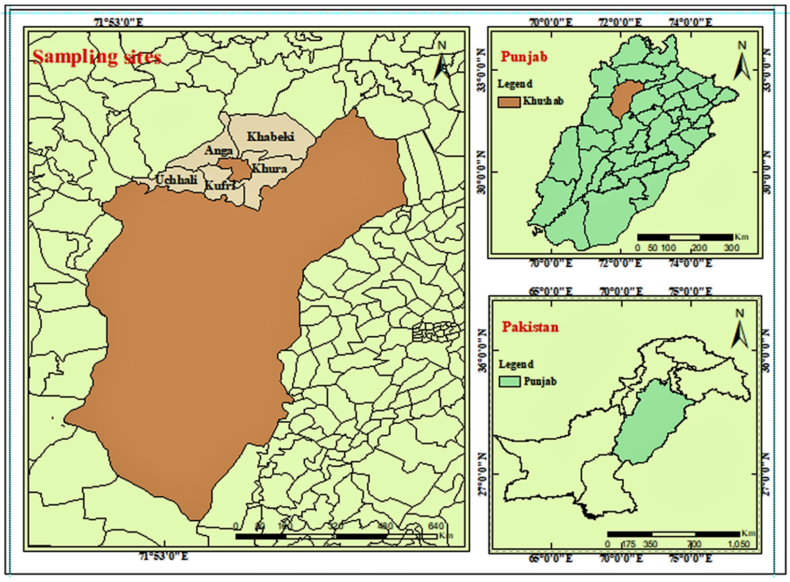

Figure 1. Locations selected for the collection of flora of Soone Valley and the surrounding salt range of Pakistan.

Table 2. Geographical coordinates of the study sites of Soone Valley (Khushab) and the surrounding salt range of Pakistan.

Localities	Latitude N	Longitude E	Elevation (m)
Anga	32.35° N	72.05° E	821
Khabbeki	32.35° N	72.12° E	774
Khura	32.23° N	72.11° E	866
Uchhali	32.56° N	72.02° E	794
Kufri	32.56° N	72.02° E	723

2.3. Bioassays with Plant Extracts

In the first bioassay, 20% methanolic plant extracts were screened against *S. frugiperda* larvae using the standard leaf-dip method. In brief, fresh cauliflower (*Brassica oleracea* L. botrytis) leaves were collected, washed with tap water and allowed to air dry for 3 min at room temperature (27 °C). Leaf discs (9 cm) were made and dipped for 30 s in 20% methanolic extracts of plants and were placed on filter paper sheets to drain out the excess solution. After drying, the treated leaf discs were placed in glass Petri plates (diameter 9 cm) lined with 2.0% agar solution to keep them fresh, and 10 pre-starved (4 h) larvae of *S. frugiperda* were released in each Petri plate. These plates were incubated in an environment chamber (Sanyo MLR-350H, Sanyo, Kyoto, Japan) under controlled conditions of 25 ± 2 °C, 60 ± 5% RH and 16 h:8 h (L:D) photoperiod. Five replicates were maintained for each treatment, and methanol-soaked leaves were used as a control. Larval mortality was examined at 12, 24, 48 and 72 h posttreatment. Furthermore, four different concentrations (i.e., 5, 10, 20 and 40%) of the three most effective botanical extracts were prepared with methanol and were bioassayed against 3rd instar larvae of *S. frugiperda* to determine their median lethal concentration (LC_{50}) and lethal time (LT_{50}) values. The bioassay protocol for this second botanical bioassay was the same as that described above.

2.4. Bioassay with Synthetic Insecticides

The comparative toxicity of synthetic insecticides against the immature *S. frugiperda* was assessed using the standard lead-dip method as described previously [24]. For this purpose, nine synthetic insecticides were purchased from authenticated pesticide dealers from the local grain market of Sargodha (Punjab, Pakistan) and were tested according to their label-recommended dose rates (Table 3). These insecticidal solutions were prepared in laboratory according to recommended dose per 80 L water as recommended for one acre coverage out in the field. Freshly prepared discs of *B. oleracea* leaves were dipped into aqueous solutions of insecticides, and after draining and drying on filter paper sheets, these discs were placed in glass Petri plates (9 cm). Ten 4 h pre-starved 3rd larvae of *S. frugiperda* were exposed to these treated leaves. Each treatment was replicated five times with water-soaked leaves acting as a control. All procedures were conducted under controlled conditions (at 25 ± 2 °C, 60 ± 5% RH and 16 h:8 h (L:D) photoperiod). Larval mortality was recorded at 12, 24, 48 and 72 h post-treatment.

Table 3. Description of synthetic insecticides bioassayed against 3rd instar larvae of the fall armyworm *Spodoptera frugiperda*.

Insecticide	Label dose (mL/Acre)	IRAC Group *	Manufacturer
abamectin	400	Avermectins	FMC, Lahore, Pakistan®
chlorantraniliprole	50	Diamides	Orange, Karachi, Pakistan®
chlorpyrifos	1000	Organophosphate	Orange, Karachi, Pakistan®
deltamethrin	80	Pyrethroid	Bayer, Karachi, Pakistan®
emamectin benzoate	200	Avermectins	Syngenta, Karachi, Pakistan®
fipronil	480	Phenylpyrazole	Orange, Karachi, Pakistan®
lambda cyhalothrin	250	Pyrethroid	FMC, Lahore, Pakistan®
lufenuron	200	Benzoylurea	Syngenta, Karachi, Pakistan®
profenofos	250	Organophosphate	Syngenta, Karachi, Pakistan®

* Insecticide Resistance Action Committee (v10.2_23March22).

2.5. Efficacy of Binary/Tertiary Mixtures

The toxicity of binary and/or tertiary mixtures of the most effective botanical and synthetic insecticide treatments was further determined using the LC_{33} and LC_{50} of botanicals and half of the label-recommended doses of synthetic insecticides. The LC_{50} values at 72 h were considered for these combination treatments. The calculation of all treatment solutions was based on previous experiments and is mentioned in Table 4. Twenty treatments, including the control, were assessed against *S. frugiperda larvae.* Here, LC_{33} concentration of each plant extract was compared alone and in tertiary combination, while LC_{50} concentrations were evaluated alone and in combination with half of the label-recommended dose rates of synthetic insecticides. All bioassay protocols were the same as those detailed above. Actual larval mortalities were compared to the expected mortalities based on the formula derived after Trisyono and Whalon [25] as follows:

For tertiary combination:

$$E = O_a + O_b\ (1 - O_a) + O_c\ (1 - O_b)$$

For binary combinations:

$$E = O_a + O_b\ (1 - O_a)$$

where E is the expected mortality for the combination and O_a, O_b and O_c are the observed mortalities of *W. somnifera*, *S. mollis* and *R. stricta* alone at a given concentration. The effect of mixtures was designated antagonistic, additive or synergistic based on χ^2 comparisons as follows:

$$\chi^2 = \frac{(O_m - E)^2}{E}$$

where O_m is the observed mortality for the binary mixture and E is the expected mortality; χ^2 with α = 0.05 was 3.84. A pair with χ^2 values > 3.84 and having greater than the expected mortality was considered to be synergistic, with χ^2 values < 3.84 representing additive effects.

Table 4. Selected effective treatments and their combinations bioassayed against 3rd instar larvae of the fall armyworm *Spodoptera frugiperda*.

Sr. No.	Botanicals or Synthetic Treatments	Concentration/Dose Used
T1	LC_{33} (*Withania somnifera*)	20%
T2	LC_{33} (*Sophora mollis*)	28%
T3	LC_{33} (*Rhazya stricta*)	29%
T4	LC_{50} (*W. somnifera*)	30.21%
T5	LC_{50} (*S. mollis*)	33.32%
T6	LC_{50} (*R. stricta*)	36.41%
T7	emamectin benzoate (1/2 of LD) *	100 mL/acre
T8	chlorpyrifos (1/2 of LD) *	500 mL/acre
T9	chlorantraniliprole (1/2 of LD) *	25 mL/acre
T10	LC_{33} (*W. somnifera* + *S. mollis* + *R. stricta*)	20% + 28% + 29%
T11	LC_{50} (*W. somnifera*) + emamectin benzoate (1/2 of LD) *	30.21% + 125 mL/acre
T12	LC_{50} (*W. somnifera*) + chlorpyrifos (1/2 of LD) *	30.21% + 500 mL/acre
T13	LC_{50} (*W. somnifera*) + chlorantraniliprole (1/2 of LD) *	30.21% + 25 mL/acre
T14	LC_{50} (*S. mollis*) + emamectin benzoate (1/2 of LD) *	33.32% + 125 mL/acre
T15	LC_{50} (*S. mollis*) + chlorpyrifos (1/2 of LD) *	33.32% + 500 mL/acre
T16	LC_{50} (*S. mollis*) + chlorantraniliprole (1/2 of LD) *	33.32% + 25 mL/acre
T17	LC_{50} (*R. stricta*) + emamectin benzoate (1/2 of LD) *	36.41% + 125 mL/acre
T18	LC_{50} (*R. stricta*) + chlorpyrifos (1/2 of LD) *	36.41% + 500 mL/acre
T19	LC_{50} (*R. stricta*) + chlorantraniliprole (1/2 of LD) *	36.41% + 25 mL/acre
T20	Control (water only)	0.00%

* LD = labeled dose.

2.6. GC–MS Characterization of Effective Plant Extracts

A GC–MS-DSQ II (Thermo Scientific, San Jose, CA, USA) with a gas chromatograph interfaced to a mass spectrometer apparatus was used to analyze the crude methanolic extracts of *W. somnifera*, *S. mollis* and *R. stricta*. The following conditions were employed: a TR-5MS fused silica capillary column (30 × 250 × 0.25 m, composed of 5% phenyl/95% dimethylpolysiloxane) operating in electron impact mode at 70 eV; helium (99.99%) was used as the carrier gas at a constant flow rate of 1 mL min^{-1}, and an injection volume of 1 µL was used (ay split ratio of 10:1); injector temperature was 240 °C, and the ion-source temperature was 200 °C. Initially, the oven temperature was adjusted to 70 °C (isothermal for 2 min) and then rose to 240 °C at a rate of 10 °C min^{-1}, followed by a 9 min isothermal at 280 °C. The mass spectra were acquired at 70 eV with a scan interval of 0.5 s with fragments ranging in size from 40–440 Da. The total time spent running the GC was 40 min [26]. The compounds were identified by comparing the GC–MS mass spectra to those in the Wiley/NIST databases [27].

2.7. Statistical Analysis

Data regarding *S. frugiperda* larval mortality were interpreted statistically using the program Statistix 8.1® (Analytical Software, Tallahassee, FL, USA). Factorial analysis of variance (ANOVA) was used to examine the mortality data, and the treatment means were compared using an honestly significant difference (HSD) post hoc test at the 95% probability level ($p \leq 0.05$). Lethal concentration 33 percent (LC_{33}), median lethal concentration (LC_{50}) and time (LT_{50}) values were calculated by probit analysis [28] using regression software IBM SPSS® (Version 20.0). Prior to probit analysis, mortality data were corrected using Abbott's formula [29] and were normalized by arcsine square root transformation (arsin (sqrt(x))) [30].

3. Results

3.1. Screening of Plant Extracts for Insecticidal Potential

Factorial analysis revealed a significant effect of both botanical treatments ($F_{11,144} = 86.55$; $p < 0.001$) and the time factor ($F_{2,64} = 164.24$; $p < 0.001$) and of their interactions ($F_{22,144} = 5.34$; $p < 0.001$) on the mortality of *S. frugiperda* larvae. The 20% methanolic extracts of *S. mollis* and *W. somnifera* showed the highest mean corrected mortality (~37%) of the 3rd instar larvae of *S. frugiperda*, whereas *R. stricta* and *O. ferruginea* extracts caused 32 and 17% mortality, respectively. The lowest mortality of *S. frugiperda* larvae was observed in the case of *B. papillosa and P. aphylla* (7%), followed by *M. arenaria* (8%), *S. moorcroftiana* (8%), *S. incanum* (8%) and *S. nigrum* (8%) (Figure 2). The toxicity of each botanical extract against *S. frugiperda* concerning the exposure time indicated that *S. mollis* and *W. somnifera* caused the highest corrected mortality (51%), followed by *R. stricta* (49%) at 72 h post-treatment. Although *M. arenaria*, *P. aphylla*, *S. moorcroftiana*, *S. incanum* and *S. nigrum* showed the least mortality (~12% each) (Supplementary Figure S1), no mortality of *S. frugiperda* was observed by *P. aphylla* at 24 h posttreatment, whereas *B. papillosa*, *M. arenaria*, *S. moorcroftiana*, *S. incanum* and *S. nigrum* caused only 2% mortality at 24 h post-treatment (Supplementary Figure S1).

For the second bioassay using different serial concentrations of botanical extracts, probit analysis revealed that the most effective botanical extract was *W. somnifera* (LC_{50} = 40.42 and 30.21% at 48 and 72 h posttreatment, respectively), followed by *S. mollis* (LC_{50} = 44.09 and 33.32%) and *R. stricta* (LC_{50} = 75.10 and 36.41%) at 48 and 72 h of application, respectively (Supplementary Table S1). In comparison, the extract of *O. ferruginea* resulted in the lowest toxicity to the 3rd instar larvae of *S. frugiperda*, with an LC_{50} value of 245.79% at 72 h post-treatment. In the case of the medial lethal time (LT_{50}) values, the 40 and 20% extracts of *W. somnifera* exhibited minimum LT_{50} values (i.e., 48.59 and 51.82 h), followed by *S. mollis* (49.06 and 52.31 h) and *R. stricta* (54.89 and 55.02 h) (Supplementary Table S2).

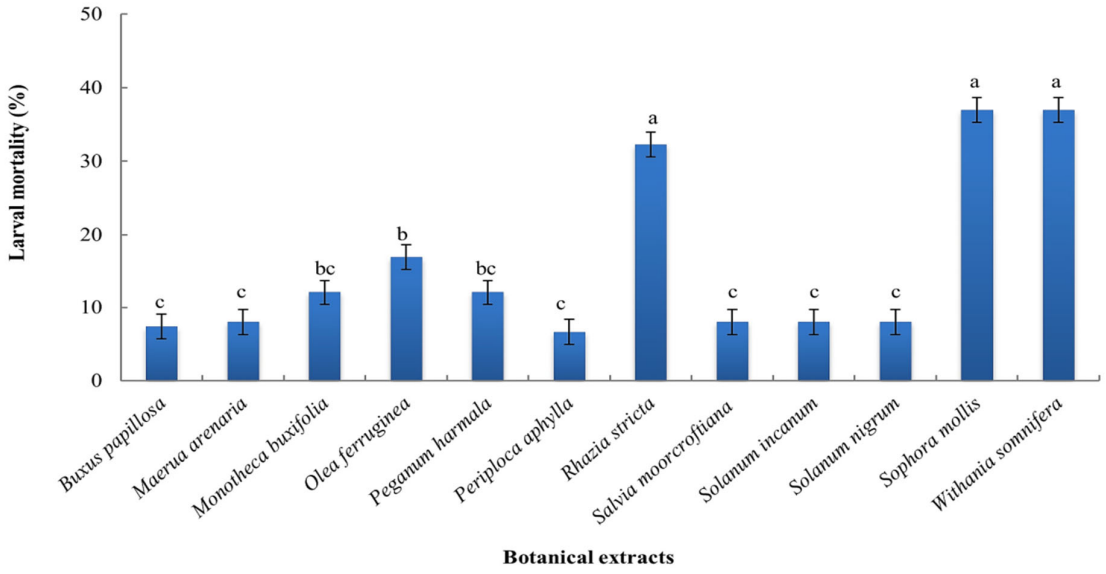

Figure 2. Percent mortality (mean ± SE; n = 10) of 3rd instar larvae of fall armyworm *Spodoptera frugiperda* at 72 h post-exposure to 20% methanolic extracts of different plant species. Alphabets at bar tops indicate significant differences among the botanical treatments (one-way ANOVA; HSD at $\alpha = 0.05$).

3.2. Toxicity of Synthetic Insecticides

The toxicity bioassay with nine synthetic insecticides against the 3rd instar larvae of *S. frugiperda* revealed a significant effect of both insecticidal treatments ($F_{8,324} = 141.81$; $p < 0.001$) and the time factor ($F_{3,324} = 710.51$; $p < 0.001$), and revealed their interactions ($F_{24,324} = 4.84$; $p < 0.001$) on the mortality of *S. frugiperda* larvae, where the label-recommended dose of emamectin benzoate showed the highest mean corrected mortality (~45%), followed by chlorpyrifos (40%) and chlorantraniliprole (38%). In comparison, lufenuron caused minimum larval mortality (~16%), followed by fipronil (17%) and lambda-cyhalothrin (18%). There was no significant difference among the larval mortalities of *S. frugiperda* caused by profenofos, abamectin and deltamethrin (i.e., 28, 28 and 27%, respectively) (Figure 3).

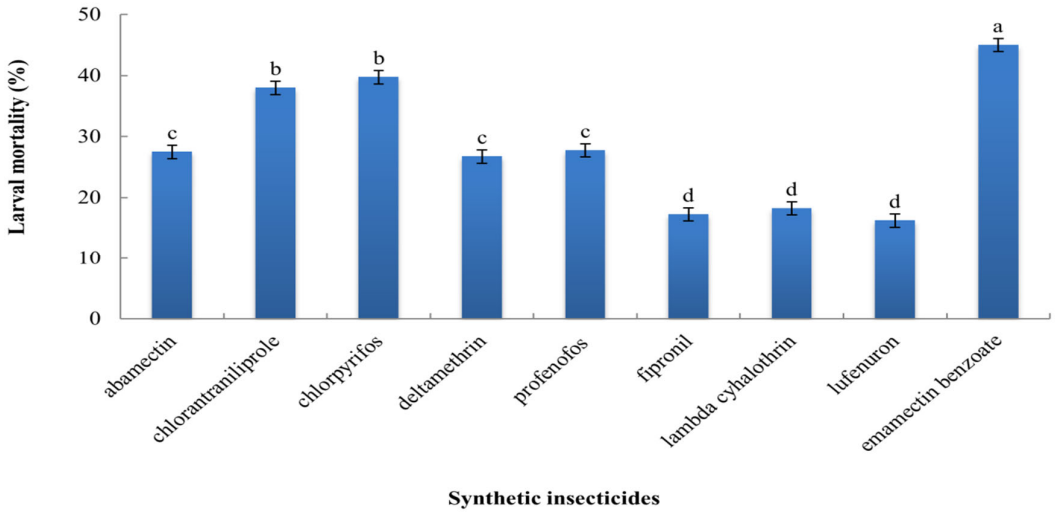

Figure 3. Percent mortality (mean ± SE; n = 10) of 3rd instar larvae of the fall armyworm *Spodoptera frugiperda* by different synthetic insecticides. Letters above the bars indicate significant differences among the treatments (one-way ANOVA; HSD at $\alpha = 0.05$).

3.3. Efficacy of Binary/Tertiary Combinations of Effective Synthetic and Botanical Treatments

Ten binary/tertiary combinations of the most effective botanical and synthetic insecticidal treatments were tested in the third bioassay. Among these combinations, seven pesticide combinations exhibited synergistic toxicity, while three combinations showed an additive effect on the mortality of 3rd instar larvae of *S. frugiperda* (Table 5). The tertiary combination of LC_{33} values of *W. somnifera*, *S. mollis* and *R. stricta* exhibited 29.3% mortality compared to an expected mortality of 65.2% and demonstrated synergistic toxicity. In all treatments, the application of individual insecticidal treatments caused lower larval mortality than their combined application. The combination of chlorpyrifos (at its half label-recommended dose) with LC_{50s} of *W. somnifera*, *S. mollis* and *R. stricta* demonstrated an observed mortality of 40.0% for each combination compared to the expected mortality of 58.3, 59.1, 59.1%, respectively. Similarly, all binary combinations of emamectin benzoate with all three botanical extracts of *W. somnifera*, *S. mollis* and *R. stricta* showed an additive effect on larval mortality (Table 5).

Table 5. Combined effect of binary/tertiary pesticidal mixtures against 3rd instar larvae of the fall armyworm *Spodoptera frugiperda*.

Pesticides (Dose)			Larval Mortality (%)						
			Pesticides			Binary/Tertiary Pesticidal Mixtures		χ^2	Effect
A	B	C	Observed A	Observed B	Observed C	Expected *	Observed		
LC_{33} (*Withania somnifera* 1)	LC_{33} (*Sophora mollis*)	LC_{33} (*Rhazya stricta*)	24.0	24.0	22.7	59.5	29.3	15.3	Synergistic
LC_{50} (*Withania somnifera*)	1/2 of LD emamectin benzoate	-	32.0	44.0	-	61.9	49.3	2.6	Additive
LC_{50} (*Withania somnifera*)	1/2 of LD chlorpyrifos	-	32.0	38.7	-	58.3	40.0	5.7	Synergistic
LC_{50} (*Withania somnifera*)	1/2 of LD chlorantraniliprole	-	32.0	36.0	-	56.5	36.0	7.4	Synergistic
LC_{50} (*Sophora mollis*)	1/2 of LD emamectin benzoate	-	33.3	44.0	-	62.7	49.3	2.8	Additive
LC_{50} (*Sophora mollis*)	1/2 of LD chlorpyrifos	-	33.3	38.7	-	59.1	40.0	6.2	Synergistic
LC_{50} (*Sophora mollis*)	1/2 of LD chlorantraniliprole	-	33.3	36.0	-	57.3	36.0	7.9	Synergistic
LC_{50} (*Rhazya stricta*)	1/2 of LD emamectin benzoate	-	33.3	44.0	-	62.7	49.3	2.8	Additive
LC_{50} (*Rhazya stricta*)	1/2 of LD chlorpyrifos	-	33.3	38.7	-	59.1	40.0	6.2	Synergistic
LC_{50} (*Rhazya stricta*)	1/2 of LD chlorantraniliprole	-	33.3	36.0	-	57.3	36.0	7.9	Synergistic

* Indicates the expected larval mortality derived from the formula of Trisyono and Whalon (1999). $\chi^2 = 3.84$ (at $\alpha = 0.05$). A combination with χ^2 value > 3.84 was considered to be synergistic, while with χ^2 value < 3.84 indicates an additive effect.

3.4. Biochemical Composition of Plant Extracts

GC–MS analysis was used to determine the presence of biologically active components in methanolic extracts of *W. somnifera*, *S. mollis*, and *R. stricta*. The major bioconstituents, their molecular weight (g mol^{-1}, M.W.), molecular formula (M.F.), retention time (s, R.T.), and peak area (%) are given in Tables 6–8, respectively. The crude extract of *W. somnifera* roots primarily comprised eighteen compounds. 1,2,4-trimethyl-benzene and 3,5-dimethyl-octane were the most abundant compounds, with areas of 9.40 and 7.34%, respectively. In comparison, the other minor compounds were present in low quantities, with relative peak areas ranging from 0.28–3.75% (Table 6). The GC–MS profile of the *S. mollis* extract revealed the presence of fifteen compounds. Among these compounds, 1-ethyl-2-methyl-benzene was the major compound with a 6.49% relative peak area, whereas the other fourteen identified compounds were recognized as minor compounds with relative peak areas ranging from 0.22–1.37% (Table 7). Chemical profiling of *R. stricta* indicated the presence of fifteen substances in its extract. The principal compounds were 1-monolinoleoylglycerol trimethylsilyl ether, decane, and lupeol with 8.73, 5.08 and 4.24% relative peak areas, respectively, while the other twelve minor compounds had relative peak areas ranging from 0.29–1.77% (Table 8).

Table 6. Chemical composition of the methanolic extract of *Withania somnifera* roots.

Peak No.	R.T.	Compounds	Area (%)	M.F.	M.W.
1	3.53	Benzene, 1,2,4-trimethyl-	9.40	C_9H_{12}	120
2	4	Octane, 3,5-dimethyl-	7.34	$C_{10}H_{22}$	142
3	4.49	Tumerone	2.09	$C_{15}H_{22}O$	218
4	6.06	Limonen-6-ol, pivalate	1.56	$C_{15}H_{24}O_2$	236
5	6.50	2-Oxazolamine, 4,5-dihydro-5-(phenoxymethyl)-N-[(phe nylamino)carbonyl]-	1.41	$C_{17}H_{17}N_3O_3$	311
6	6.83	12,15-Octadecadiynoic acid, methyl Ester	1.40	$C_{19}H_{30}O_2$	290
7	7.40	Dodecane	3.05	$C_{12}H_{26}$	170
8	7.81	(2-Aminocyclohexyl)-phenyl-methanol	0.62	$C_{13}H_{19}NO$	205
9	8.81	Pyridine, 2-(1H-tetrazol-5-yl)-	2.21	$C_6H_5N_5$	147
10	9.97	2-Vinyl-9-[3-deoxy-á-d-ribofuranosyl]hypoxanthine	0.47	$C_{12}H_{14}N_4O_4$	278
11	12.33	Bicyclo [4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	0.73	$C_{15}H_{24}O_2$	236
12	14.25	2-Oxazolamine, 4,5-dihydro-5-(phenoxymethyl)-N-[(phenylamino)carbonyl]-	0.44	$C_{17}H_{17}N_3O_3$,	311
13	16.02	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.28	$C_{28}H_{48}O$	400
14	18.67	Cystathionine, bis(triemthylsilyl) ester	2.05	$C_{13}H_{30}N_2O_4SSi_2$	366
15	20.66	Dihydroxanthin	1.37	$C_{17}H_{24}O_5$	308
16	22.41	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	1.16	$C_{27}H_{52}O_4Si_2$	496
17	24.33	1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	2.03	$C_{13}H_{21}N_3O_3$	267
18	26.94	Cyclotrisiloxane, hexamethyl-	3.75	$C_6H_{18}O_3Si_3$	222

R.T., Area (%), M.F., and M.W., indicates the retention time, peak area, molecular formula and molecular weight.

Table 7. Chemical composition of the methanolic extract of *Sophora mollis* leaves and flowers.

Peak No.	R.T.	Compounds	Area (%)	M.F.	M.W.
1	3.59	Benzene, 1-ethyl-2-methyl-	6.49	C_9H_{12}	120
2	5.20	1-Hexadecanol, 2-methyl-	1.20	$C_{17}H_{36}O$	256
3	6.93	E-9-Tetradecenoic acid	1.37	$C_{14}H_{26}O_2$	226
4	8.09	Pregnane-3,11,20,21-tetrol, cyclic 20,21-(butyl boronate), (3à,5á,11á,20R)-	1.28	$C_{25}H_{43}BO_4$	418
5	9.93	Oxirane, hexadecyl-	0.57	$C_{18}H_{36}O$	268
6	11.82	Naphthalene, 1,1′-(1,10-decanediyl)bis-	0.43	$C_{30}H_{34}$	394
7	13.80	Tetraethylrhodamine	0.95	$C_{28}H_{31}N_2O_3$	443
8	15.26	1-Oxaspiro [4.4]non8-ene-4,7-dione, 9-hydroxy-6-(3-methyl-2-butenyl)-2-(1-methylethyl)-8-(3-methyl-1-oxobutyl)-	0.26	$C_{21}H_{30}O_5$	362
9	16.61	10-Hydroxy-5,7-dimethoxy-2,3-dimethyl-1,4-anthracenedione	0.38	$C_{18}H_{16}O_5$	312
10	18.58	7,8,12-Tri-O-acetylingol	0.74	$C_{26}H_{36}O_9$	492
11	19.40	Digitoxin	0.27	$C_{41}H_{64}O_{13}$	764
12	20.74	Z-10-Methyl-11-tetradecen-1-ol Propionate	0.75	$C_{18}H_{34}O_2$	282
13	23.09	Isoproturon	0.51	$C_{12}H_{18}N_2O$	206
14	24.02	1-Monolinoleoylglycerol trimethylsilyl Ether	0.22	$C_{27}H_{54}O_4Si_2$	498
15	27.30	Silane, 1,4-phenylenebis-trimethyl-	0.52	$C_{12}H_{22}Si_2$	2221

R.T., Area (%), M.F., and M.W., indicates the retention time, peak area, molecular formula and molecular weight.

Table 8. Chemical composition of the methanolic extract of *Rhazya stricta* leaves and flowers.

Peak No.	R.T.	Compounds	Area (%)	M.F.	M.W.
1	4.04	Decane	5.08	$C_{10}H_{22}$	142
2	5.73	Octane, 5-ethyl-2-methyl-	1.18	$C_{11}H_{24}$	156
3	6.14	Palmitic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl Ester	0.29	$C_{26}H_{42}O_4$	418
4	9.56	2-Propenoic acid, tridecyl ester Acetic acid	0.65	$C_{16}H_{30}O_2$	254
5	10.48	17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16, 17-dodecahydro-1H-cyclopenta[a]phenanthren-11-yl (ester)	1.77	$C_{25}H_{34}O_5$	414
6	11.62	Stearic acid, 3-(octadecyloxy)propyl ester	0.54	$C_{39}H_{78}O_3$	594
7	12.31	Methyl abietate isomer	0.61	$C_{21}H_{32}O_2$	316
8	14.31	2,5-Octadecadiynoic acid, methyl ester	1.19	$C_{19}H_{30}O_2$	290
9	16.69	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	0.54	$C_{18}H_{30}O_2$	278
10	17.24	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	1.01	$C_{35}H_{68}O_5$	568
11	19.01	Akuammilan-16-carboxylic acid, 17-(acetyloxy)-, methyl ester, (16R)-	0.64	$C_{23}H_{26}N_2O_4$	394
12	20.46	à-N-Normethadol	1.75	$C_{20}H_{27}NO$	297
13	22.19	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	1.06	$C_{27}H_{52}O_4Si_2$	496
14	24.90	1-Monolinoleoylglycerol trimethylsilyl ether	8.73	$C_{27}H_{54}O_4Si_2$	498
15	26.98	Lupeol	4.24	$C_{30}H_{50}O$	426

R.T., Area (%), M.F., and M.W., indicates the retention time, peak area, molecular formula and molecular weight.

4. Discussion

This research work revealed the synergistic effect of some promising indigenous plant extracts and commonly used synthetic insecticides on the 3rd instar larvae of *S. frugiperda*. An initial screening bioassay performed with 20% methanolic extracts of twelve indigenous plant species demonstrated that the extracts of *S. mollis*, *W. somnifera* and *R. stricta* were the most effective botanicals exhibiting the highest mortality of *S. frugiperda* larvae. Our results corroborate the findings of some recent studies that have demonstrated the toxicity of acetone extracts of indigenous plant species of the same study area against the termite (*Odontotermes obesus*), mosquito (*Culex quinquefasciatus*), psyllid (*Diaphorina citri*) and armyworm (*S. litura*) [20,21,31]. Similarly, Phambala et al. [32] demonstrated the insecticidal activity of 10% methanolic extracts of some indigenous ethnomedicinal plants of Mitundu (Malawi) against *S. frugiperda* larvae. They showed that extracts of *Nicotiana tabacum* and *Lippia javanica* caused significantly higher mortality (62–66%) of *S. frugiperda* larvae. Moreover, Rioba and Stevenson [33] reviewed a number of previous studies documenting significant larvicidal and ovicidal activity of local plant extracts against *S. frugiperda*.

Our second feeding bioassay conducted using different concentrations of these plant extracts revealed *W. somnifera*, *S. mollis* and *R. stricta* as the most effective botanical treatments, with minimum LC_{50} values of 30.21, 33.32 and 36.41% at 72 h of application, respectively, and minimum LT_{50} values of 48.59, 49.06 and 54.89 h by 40% extracts, respectively. Although these three plant species are not well studied regarding their insecticidal potential, many phytoextracts, including plant extracts and essential oils, have been demonstrated to show larvicidal [21,33–37], ovicidal and other anti-insect activities [32,36] against *S. frugiperda* and other *Spodoptera* species. Our results are in line with Gupta and Srivastava [38], who showed significant mortality (63.33%) of *Callosobruchus chinensis* adults by 10% ethanolic extracts of *W. somnifera* roots. Similarly, extracts of *S. mollis* (and other plants from the genus *Sophora*) and *R. stricta* have been known to contain many plants secondary metabolites with allelopathic, antibiotic, nematicidal and insecticidal potential [39,40].

The results of the third bioassay with commonly used synthetic insecticides revealed emamectin benzoate, chlorpyrifos and chlorantraniliprole as the most effective insecticides against *S. frugiperda* larvae. Many previous studies based on socioeconomic surveys and laboratory and field bioassays have demonstrated the effectiveness of emamectin benzoate, chlorpyrifos and chlorantraniliprole against different species of *Spodoptera* [41,42]. Emamectin benzoate is an effective insecticide that is effectively used alone or in combination with other insecticides in East Africa and South Asia. For instance, 92% and 88% of farmers from Rwanda and Uganda utilized a combination of emamectin benzoate and cypermethrin against fall armyworm, respectively [43]. Shallot growers in Java (Indonesia) use chlorpyrifos for effective control of *S. exigua* [44], whereas some studies have indicated the effectiveness of chlorantraniliprole against the 3rd instar larvae of *S. litura* [45,46]. A recent study was reported on the efficacy of abamectin and broflanilide belong to the avermectin and diamides group cause significant mortality of 87.3 and 91.3% against second instar *S. frugiperda* larvae at 72 h post-treatment in China [47].

In the fourth bioassay, combinations of LC_{33} and LC_{50} of the most effective botanicals (*R. stricta*, *S. mollis* and *W. somnifera*) and half of the labeled dose of synthetic insecticides (emamectin benzoate, chlorpyrifos and chlorantraniliprole) were assessed against 3rd instar larvae of *S. frugiperda*. Among the ten combinations, seven exhibited synergy, and three produced additive toxicity against *S. frugiperda*. Our findings regarding the synergistic and additive effects of different botanical and synthetic insecticides are consistent with those of many previous studies. For instance, our results are consistent with those of Fazolin et al. [48], who revealed a synergistic toxicity of beta-cypermethrin and fenpropathrin against *S. frugiperda* by combining with *Piper aduncum* essential oil. Similarly, binary combinations of different phyto-constituents (α-thujone, (+)-camphor, 1,8-cineole, and α-caryophyllene) from *Salvia hispanica* exhibited synergistic toxicity against *S. exigua* [49]. The insecticidal activity of garlic and thymol oils is enhanced against *S. littoralis* when combined with cypermethrin and chlorpyrifos [50]. Similarly, Rao and Dhingr [51], and

Ruttanaphan et al. [52] revealed synergistic and additive activity of different vegetable and plant essential oils along with cypermethrin against larvae of *S. litura*. Similarly, Silva et al. [53] reported significantly higher mortality of 3rd instar larvae of *S. frugiperda* by the binary combinations of LD_{50} doses of pyrethroid deltamethrin and *Ocimum basilicum*-derived linalool oil.

5. Conclusions

Based on the overall study results, it is concluded that the methanolic extracts of *R. stricta*, *S. mollis*, and *W. somnifera* exhibited significant toxicity potential against fall armyworm larvae. The combination of LC_{33s} and LC_{50s} of these plant extracts along with half of the label-recommended doses of chlorpyrifos, chlorantraniliprole and emamectin benzoate synergized the toxicity against 3rd instar larvae of *S. frugiperda*, suggesting their potential incorporation into future integrated pest management of *S. frugiperda*. Nevertheless, field evaluation of these botanical and synthetic insecticidal combinations regarding their effect on *S. frugiperda* and its natural enemies (insect predators and parasitoids) constitute future perspectives of this study.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12061289/s1, Table S1: Median lethal concentration (LC_{50}) values of different botanicals evaluated against 3rd instar larvae of fall armyworm *Spodoptera frugiperda*; Table S2: Median lethal time (LT_{50}) values of different plant extracts evaluated against 3rd instar *Spodoptera frugiperda* larvae. Figure S1: Corrected percent mortality (mean ± SE; $n = 10$) of 3rd instar larvae of fall armyworm *Spodoptera frugiperda* by 20% methanolic extracts of different plant species recorded at different time intervals. Alphabets at bar tops indicate statistical difference among each botanical treatments at different time intervals (one-way ANOVA; HSD at $\alpha = 0.05$).

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