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Original article

Laboratory evaluation of selected botanicals and insecticides against invasive *Spodoptera frugiperda* (Lepidoptera: Noctuidae)Atif Idrees^{a,b}, Ayesha Afzal^{a,c}, Talha Ali Chohan^c, Sikandar Hayat^c, Ziyad Abdul Qadir^{d,e}, Abdel-Rhman Z. Gaafar^f, Ali Tan Kee Zuan^g, Jun Li^{a,*}^a Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou 510260, China^b Guizhou Provincial Key Laboratory for Agricultural Pest Management of the Mountainous Region, Scientific Observing and Experimental Station of Crop Pest in Guiyang, Ministry of Agriculture and Rural Affairs, Institute of Entomology, Guizhou University, Guiyang 550025, China^c Institute of Molecular Biology and Biotechnology, The University of Lahore, 1-Km Defense Road, Lahore 54000, Pakistan^d Honeybee Research Institute, National Agricultural Research Centre, Park Road, Islamabad 45500, Pakistan^e Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE 19716, USA^f Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 11451, Riyadh, Saudi Arabia^g Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia

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ABSTRACT

Background: Maize is an economical crop of China, and its production has been severely affected by the invasive *Spodoptera frugiperda* in recent year. Application of synthetic pesticides are one of the most effective practices against FAW as an emergency control. This pest causes serious damage to the maize crop worldwide in recent decade, especially in China.

Methods: To find an alternative to synthetic insecticides there were total 16 different chemicals were used including ten botanical insecticides comprising of seven botanicals, azadirachtin, pyrethrin, nicotine, osthole, rotenone, *Celastrus angulatus*, matrine, and three insect growth regulators, diflubenzuron, lufenuron and buprofezin. Six synthetic insecticides, including emamectin benzoate, indoxacarb, imidacloprid and thiamethoxan, chlorantraniliprole, chlorfenapyr, were evaluated against 2nd instar of *S. frugiperda* larvae using leaf-dip method.

Results: The results revealed that osthole, azadirachtin, buprofezin and pyrethrin were showed the significant larval mortality of 98.0, 96.7, 94.0 and 90.7%, in 120 h observation and exhibiting minimum LC₅₀ (39.04, 35.58, 61.45 and 48.46 mg/L, respectively) and LT₅₀ (48.91, 68.85, 58.67 and 58.57 h, respectively) values. Among tested synthetic insecticides, emamectin benzoate, chlorantraniliprole and chlorfenapyr were showed significant higher mortality against larvae of *S. frugiperda* (99.3, 96.0 and 89.3%, respectively) in 72 h observation by exhibiting minimum LC₅₀ (0.26, 0.39 and 0.72 mg/L, respectively) and and LT₅₀ (10.18, 10.57 and 13.42 h, respectively) values. More study is needed to test the laboratory findings in the field, although the efficient biorational pesticides might be utilized as part of integrated pest management against *S. frugiperda*.

Conclusion: The effective chemicals could be used in the management for *S. frugiperda*. The highest discriminating concentrations of tested botanical insecticides, insect growth regulators and insecticides caused significant mortality of *S. frugiperda* larvae.

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1. Introduction

The United States is the leading producer and consumer of maize, but China has quickly overtaken them (Wu et al., 2021). *Spodoptera frugiperda*, fall armyworm (FAW) is native to the Americas and extremely prolific, polyphagous pest of more than 350 different plant species especially of maize (Navik et al., 2021). *S. frugiperda* has expanded throughout the world, with first detection in Africa, Nepal, Indonesia, and Swaziland (Acharya et al., 2020). The production of horticultural crop is severely threatened due to attack of FAW, which has been found in cereals and vegetables around the world (Yeboah et al., 2021).

One survey predicts that by 2019, FAW would cause annual financial losses of US\$2.5–6.2 billion in maize across 44 African nations (Bengyella et al., 2021). Hence, FAW is classified as an A1 quarantine invasive pest (Kasoma et al., 2020). The *S. frugiperda* caused major yield losses of maize in Ethiopia and Kenya and most of the maize growers preferred to use pesticides for controlling this pest (Susanto et al., 2021a). On December 11th, 2018, the FAW corn strain was first identified in China (Guo et al., 2018), damaging maize, in Yunnan province, China, and afterwards invaded 26 provinces (Jing et al., 2020). At the seedling and flowering phases, FAW larvae caused 78 and 65% of the damage in peanut fields (Yang X et al., 2019), 30 to 90% of the damage in wheat fields (Yang et al., 2020). When the population of FAW is very high, it may also cause harm to tobacco crops (Xu et al., 2019).

The creation of integrated pest management is only one example of the many environmentally friendly methods of pest management that have been the subject of studies across the world (Idrees et al., 2021, 2017; Qadir et al., 2021). Although the control of these insect pests with insecticides remains until today the most serious concern. However, it is often ineffective, and the frequent misuse of these insecticides has led to the emergence of insect resistance to several classes of insecticides (Goergen et al., 2016). The selected botanical insecticides, insect growth regulators and novel insecticides might play a significant role in the management of FAW.

In order to determine the most effective botanicals and synthetic insecticides that may be advised against FAW, the current study was done to evaluate the effectiveness of various botanical and synthetic insecticides against 2nd instar larvae of FAW under laboratory circumstances.

2. Materials and methods

2.1. Insects

During the growing season for maize in Guangdong province, China, larvae were collected and used to construct a colony of *S. frugiperda*. Adults were artificially fed a 10% honey solution soaked in sterile cotton balls while the captured larvae were raised on fresh maize leaves. Neonates of *S. frugiperda* were transferred to a box (28 × 17 × 18 cm) from a development chamber maintained at 25 ± 2 °C, with a photoperiod of 16:8h (L: D), and 65% 5% R.H. All the experiment was conducted at Institute of Zoology, Guangdong Academy of Sciences. A colony of larvae was maintained for one hundred generations in an artificial indoor environment.

2.2. Botanical and synthetic insecticidal treatments

We put ten widely used botanicals and six widely available synthetic insecticides from five chemical classes through their paces against 2nd instar *S. frugiperda*. You may find the formulation type and supplier details at (Table 1).

2.3. Toxicity bioassays with botanical and synthetic insecticides

The typical leaf-dip approach was used to carry out the experiment. In contrast to first instar larvae, which are fragile and sensitive and susceptible to mechanical harm when handled, second instar larvae are utilized because they are simpler to handle or manipulate. For each botanical and synthetic insecticides, five different concentrations were prepared in distilled water by serial dilution from a stock solution i.e., (C1–C5) 400, 200, 100, 50, 25 mg/L and (3.125, 6.25, 12.5, 25.0, 50.0 mg/L), respectively. Three newly made discs of maize leaves were put in a Petri dish after being dipped into a solution containing natural and synthetic pesticides. Each Petri plate contained thirty *S. frugiperda* 2nd instar larvae that had been pre-starved for four hours. All bioassays were conducted and maintained chamber at 25 ± 2 °C, with a photoperiod of 16 h:8h (L: D) and 65% ± 5% RH. Larval mortality was recorded 24, 48, 72, 96 and 120 h post-treatment for botanicals while 24, 48 and 72 h post-treatment for synthetic insecticides. Larvae were regarded alive if they moved in response to the gentle brushing of a camel's hair, and dead if they did not respond. A total

Table 1
Information about botanical and synthetic insecticides tested against 2nd instar *S. frugiperda*.

Active Ingredients	Formulation	Chemical Group	Source	Manufacturer
Botanicals				
Azadirachtin	25% WP	Limonoid Group	Neem tree	Dow AgroSciences
Pyrethrin	70% WP	Organic Compounds	Chrysanthemum flowers	Dow AgroSciences
Nicotine	1% LC	Alkaloid	Tobacco Plant	Jiangsu Yangnong Chemicals
Matrine	40% WP	Alkaloid	<i>Sophora flavescens</i> Aiton	Yangjiang Company
Osthole	0.1% LC	Coumarin Compound	<i>Cnidium monnieri</i>	Beijing Kefa Weiye Chemicals
Rotenone	2% LC	Rotenones	Common Mullein	Syngenta
<i>C. angulatus</i>	99% WP	Sesquiterpene polyol esters	Root bark of <i>C. angulatus</i>	Chengdu Newsun Crop Science Co., Ltd.
Diflubenzuron	0.1 LC	Insect growth regulators		Syngenta
Lufenuron	25% WG	IGRs		Syngenta
Buprofezin	25% WP	IGRs		Syngenta
Synthetic Insecticides				
Emamectin benzoate	5% ME	6A, Avermectins		Hebei Weiyuan Company
Indoxacarb	150 EC	22A, Oxadiazines		Hebei Weiyuan Company
Imidacloprid	600 SC	4A, neonicotinoids		Hebei Weiyuan Company
Thiamethoxam	25% WG			
Chlorantraniliprole	5% SC	28A, Diamides		DuPont, USA
Chlorfenapyr	30% EC	13A, Pyrroles		Fuyang Chemicals

WP: wettable powder; LC: liquid concentrations; WG: water soluble granule; SC: suspension concentrate; ME: microemulsion; EC: emulsifiable concentrate.

of 150 larvae (five replicate/30 larvae in each) were used in each treatment. Fresh leaves treated with distilled water was used in the control treatment.

$$\text{Toxicity Index} = \frac{\text{LC}_{50} \text{ of the most effective compound}}{\text{LC}_{50} \text{ of the other tested compound}} \times 100$$

2.4. Statistical analysis

The POLO Plus program was used to examine the bioassay results (Robertson et al., 1980), determine the median lethal concentrations (LC₅₀), 95% confidence limits (CLs), slope, standard error, and chi-squared (χ²) test. The SPSS statistics software was used to determine the degree of freedom (df) and p value. Data on larval mortality were analyzed using factorial analysis of variance (ANOVA), with mortality rates corrected using the Abbott technique (Abbott, 1925), and the treatment means compared using Tukey’s highly significant difference (HSD) post-hoc test at the 95% level of significance. Two LT₅₀ values were considered to be statistically distinct if their 95% confidence intervals did not overlap (Litchfield and Wilcoxon, 1949). The percentages of larval mortality for each treatment were transformed using an arcsine function to lessen the amount of variability in the data (Freeman et al., 1985). In contrast to factorial analysis of variance, which was used to calculate mortality data, one way analysis of variance was used to compute mortality data.

3. Results

3.1. Comparative toxicity of botanicals against *S. frugiperda* larvae

Factorial analysis revealed that osthole, azadirachtin and buprofezin showed the highest larval mortality of 98.0, 96.7 and 94.0% respectively, followed by pyrethrin (90.7%) and diflubenzuron (82.0%). Lowest larval mortality was observed in matrine (50.7%) and *C. angulatus* (40.0%) treated with highest concentration 1 (F₁₀ = 57.06, p < 0.000) at 120 h after application (Fig. 1a).

Azadirachtin was the most toxic botanical exhibiting minimum LC₅₀ value (575.69 mg/L) followed by nicotine (1059.23 mg/L) and pyrethrin (1271.14 mg/L) against 2nd instar larvae of *S. frugiperda* mortality at 24 h post-treatment. Matrine and *C. angulatus* were found to be least effective with maximum LC₅₀ values (Table 2). Azadirachtin, osthole and pyrethrin outperformed among all tested botanicals by 604.88, 682.86 and 761.60 mg/L followed by the diflubenzuron (1030.36 mg/L) and buprofezin (1149.38 mg/L). The rotenone (1193.26 mg/L) and matrine (1301.41 mg/L) against

2nd instar larvae of *S. frugiperda* at 48 h after application of treatment (Table 2).

The osthole and azadirachtin showed LC₅₀ values 179.28 and 199.19 mg/L, followed by diflubenzuron (328.97 mg/L) and rotenone (351.14 mg/L) against 2nd instar larvae of *S. frugiperda*. The buprofezin and pyrethrin showed LC₅₀ values 360.54 and 390.61 mg/L, respectively, at 72 h after application (Table 2).

The azadirachtin and osthole showed LC₅₀ values 63.50 and 66.31 mg/L, respectively, followed by pyrethrin (83.39 mg/L) against 2nd instar larvae of *S. frugiperda*. The buprofezin and diflubenzuron showed LC₅₀ values 84.63 and 142.33 mg/L respectively, at 96 h after application (Table 3).

The buprofezin, azadirachtin and osthole exhibited 29.45, 35.58 and 39.04 mg/L, respectively, followed by pyrethrin (48.46 mg/L) against 2nd instar larvae of *S. frugiperda*. The diflubenzuron showed LC₅₀ value 61.45 mg/L respectively, followed by lufenuron (111.38 mg/L) (Table 3).

Probit regression analysis revealed that LT₅₀ values were time dependent and independent of concentration for all tested botanicals for causing mortality against 2nd instar larvae of *S. frugiperda*. The buprofezin and azadirachtin were fast acting botanical with maximum LT₅₀ values of 129.36 h (97.93–753.96) and 130.38 h (119.26–147.60), respectively, followed by osthole 138.06 h (123.12–162.49), pyrethrin 144.62 h (136.45–160.20) and diflubenzuron 169.57 h (145.68–216.90) against 2nd instar *S. frugiperda* while other botanicals were exhibiting LT₅₀ values of 181.31 (153.08–244.72) to 385.72 h (244.00–1122.95) treated with the lowest concentration 1 (25 mg/L).

Azadirachtin and osthole were exhibiting minimum LT₅₀ values of 48.40 h (36.19–59.95) and 48.91 h (35.32–61.72), respectively, followed by pyrethrin 58.57 h (41.64–77.33), buprofezin 58.67 h (35.96–84.90) and diflubenzuron 65.17 (60.39–70.24) against 2nd instar *S. frugiperda* while other botanical insecticides were exhibiting minimum LT₅₀ values of 70.03 (64.93–75.59) to 171.52 h (139.88–236.49) (Table 4).

3.2. Toxicity of insecticides against 2nd instar larvae of *S. frugiperda*

Factorial analysis revealed that emamectin benzoate, chlorantraniliprole and chlorfenapyr were showed the highest larval mortality of 99.3, 96.0 and 89.3% respectively, followed by indoxacarb (85.3%) treated with highest concentration 1 (F₆ = 132.38, p < 0.000) at 72 h post-treatment. emamectin benzoate, chlorantraniliprole and chlorfenapyr were showed the larval mortality of 57.3, 50.0 and 54.7% respectively at 72 h after application of treatment (Fig. 1b).

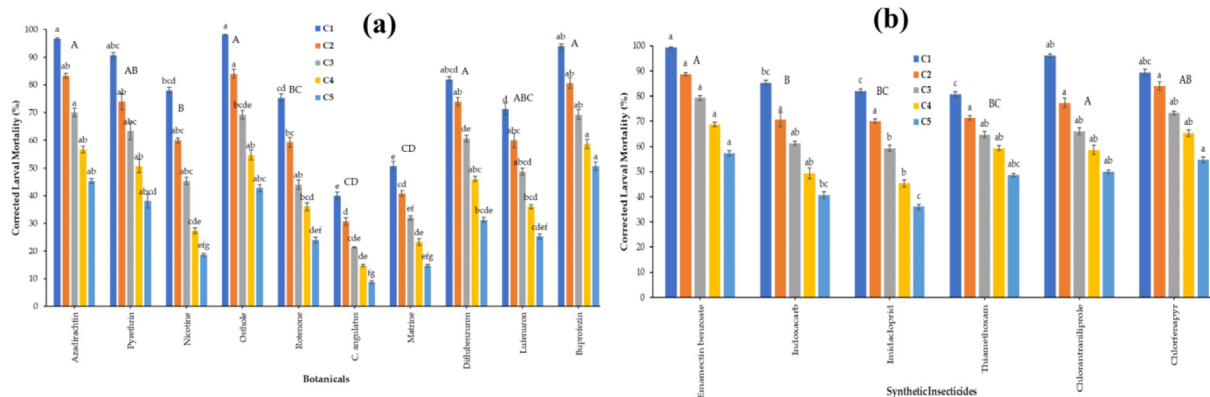


Fig. 1. Percent mortality (mean ± S.E.) of 2nd instar larvae of *S. frugiperda* against different Concentrations C1 – C5 were 400 to 25 mg/L of botanical insecticides (a) and synthetic insecticides (b). The small letters at bar tops indicate significant difference among concentrations, while capital letters indicate overall significant difference among the botanical insecticides (factorial ANOVA; HSD post-hoc test at α = 0.05).

Table 2
The LC₅₀ values for selected botanical tested against 2nd instar larvae of *S. frugiperda* at 24, 48 and 72 h post-treatment.

24 h post-treatment						
Botanical Insecticides	Slope ± SE	χ ² (df = 3)	P value	LC ₅₀ (95% CL) (mg/L)	Reg. equation (y = a + bx)	Toxicity index
Azadirachtin	1.31 ± 0.23	0.34	0.95	575.69 (292.30–2202.54) ^a	–3.53 + 1.26 x	100.00
Pyrethrin	0.97 ± 0.20	0.13	0.99	1271.14 (455.75–13102.88) ^{bc}	–2.98 + 0.95 x	45.29
Nicotine	1.18 ± 0.25	0.97	0.81	1059.23 (405.30–10643.33) ^{ab}	–3.36 + 1.06 x	54.35
Osthole	0.10 ± 0.19	0.00	1.00	4235.25 (1645.81–33278.17) ^{cd}	–3.61 + 1.00 x	13.59
Rotenone	1.02 ± 0.25	1.63	0.65	8092.34 (223.10–315354.49) ^{fgh}	–3.69 + 0.90 x	7.11
<i>C. angulatus</i>	1.16 ± 0.33	0.41	0.94	8380.47 (2122.56–1018209.58) ^{ghj}	–4.26 + 1.04 x	6.87
Matrine	0.98 ± 0.24	0.02	0.10	8888.55 (2378.67–357704.09) ^{ghij}	–3.85 + 0.97 x	6.48
Diflubenzuron	1.11 ± 0.23	0.09	0.99	4752.70 (1747.74–50637.02) ^{de}	–4.02 + 1.08 x	12.11
Lufenuron	1.10 ± 0.28	0.95	0.81	7560.38 (2126.55–321239.07) ^{fg}	–3.81 + 0.91 x	7.61
Buprofezin	0.10 ± 0.20	0.33	0.95	4905.20 (1791.36–47763.10) ^{ef}	–3.57 + 0.95 x	11.74
48 h post-treatment						
Azadirachtin	1.40 ± 0.15	0.54	0.91	604.88 (449.37–929.37) ^a	–3.79 + 1.35 x	100.00
Pyrethrin	1.46 ± 0.17	0.53	0.91	761.60 (546.98–1252.72) ^{bc}	–4.09 + 1.40 x	79.42
Nicotine	0.91 ± 0.16	0.11	0.99	2898.64 (1281.38–14740.52) ^{ghi}	–3.11 + 0.89 x	20.87
Osthole	1.25 ± 0.14	4.51	0.21	682.86 (484.26–1141.10) ^{ab}	–3.38 + 1.18 x	88.58
Rotenone	1.16 ± 0.16	0.03	0.10	1193.26 (734.86–2669.80) ^{def}	–3.56 + 1.15 x	50.69
<i>C. angulatus</i>	1.07 ± 0.21	0.29	0.96	4095.46 (1614.28–32331.87) ^{ghij}	–4.01 + 1.14 x	14.77
Matrine	1.15 ± 0.16	0.25	0.97	1301.41 (782.49–3067.78) ^{ef}	–3.53 + 1.12 x	46.48
Diflubenzuron	1.24 ± 0.16	0.10	0.99	1030.36 (667.54–2074.74) ^{cd}	–3.79 + 1.26 x	58.71
Lufenuron	1.16 ± 0.17	0.12	0.99	1370.84 (813.54–3340.54) ^{fgh}	–3.69 + 1.18 x	44.12
Buprofezin	1.22 ± 0.16	0.46	0.93	1149.38 (723.24–2457.42) ^{de}	–3.64 + 1.18 x	52.62
72 h post-treatment						
Azadirachtin	1.49 ± 0.13	1.68	0.64	199.19 (169.20–241.37) ^{ab}	–3.37 + 1.47 x	90.00
Pyrethrin	1.42 ± 0.14	0.21	0.98	390.61 (310.28–532.07) ^{def}	–3.67 + 1.41 x	45.90
Nicotine	1.13 ± 0.14	1.28	0.73	730.15 (498.57–1314.08) ^{fgh}	–3.14 + 1.09 x	24.55
Osthole	1.05 ± 0.12	1.01	0.80	179.28 (144.58–233.57) ^a	–2.36 + 1.05 x	100.00
Rotenone	1.35 ± 0.13	0.94	0.82	351.14 (279.51–475.87) ^{cd}	–3.38 + 1.32 x	51.06
<i>C. angulatus</i>	1.08 ± 0.18	0.18	0.98	2457.27 (1193.70–9902.21) ^{ghij}	–3.74 + 1.12 x	7.30
Matrine	1.12 ± 0.14	0.32	0.96	825.65 (547.91–1576.44) ^{fghi}	–3.26 + 1.12 x	21.71
Diflubenzuron	1.19 ± 0.13	0.46	0.93	328.97 (257.39–459.06) ^c	–3.02 + 1.20x	54.50
Lufenuron	1.24 ± 0.13	0.89	0.83	456.57 (345.70–676.70) ^{efg}	–3.37 + 1.27 x	39.27
Buprofezin	0.97 ± 0.12	0.02	0.10	360.94 (266.04–563.88) ^{cde}	–2.47 + 0.97 x	49.67

SE, χ², df, CL and mg/L indicate standard error, chi-square, degrees of freedom, confidence limits and milligrams per liter, respectively. When the 95% confidence intervals for two or more LC₅₀ values for the same set of tested plants do not overlap (P 0.05), the results are statistically different.

Table 3
LC₅₀ values for selected botanical tested against 2nd instar larvae of *S. frugiperda* at 96 and 120 h post-treatment.

96 h post-treatment						
Botanical insecticides	Slope ± SE	χ ² (df = 3)	P value	LC ₅₀ (95% CL) (mg/L)	Reg. equation (y = a + bx)	Toxicity Index
Azadirachtin	1.44 ± 0.12	3.29	0.35	63.50 (53.21–74.34) ^a	–2.66 + 1.48 x	100.00
Pyrethrin	1.03 ± 0.11	0.81	0.85	83.39 (66.77–102.59) ^{abc}	–2.00 + 1.04 x	76.15
Nicotine	1.27 ± 0.12	0.75	0.86	271.70 (219.89–356.95) ^{fg}	–3.06 + 1.26 x	23.37
Osthole	1.47 ± 0.12	4.03	0.26	66.31 (55.96–77.31) ^{ab}	–2.75 + 1.51 x	95.33
Rotenone	1.20 ± 0.12	0.30	0.96	201.62 (165.39–257.30) ^{ef}	–2.77 + 1.20 x	31.49
<i>C. angulatus</i>	1.01 ± 0.14	0.22	0.97	1103.67 (666.27–2577.13) ^{hi}	–3.05 + 1.00 x	5.75
Matrine	0.85 ± 0.12	0.69	0.87	720.18 (452.04–1579.29) ^h	–2.47 + 0.87 x	8.82
Diflubenzuron	1.15 ± 0.12	0.01	1.00	142.33 (117.83–176.10) ^e	–2.48 + 1.15x	44.61
Lufenuron	1.07 ± 0.11	0.38	0.94	218.04 (173.95–291.70) ^{efg}	–2.52 + 1.08 x	29.12
Buprofezin	1.27 ± 0.12	1.30	0.73	84.63 (70.65–100.46) ^{bcd}	–2.44 + 1.27 x	75.03
120 h post-treatment						
Azadirachtin	1.42 ± 0.13	5.74	0.12	35.58 (27.75–43.19) ^{ab}	–2.45 + 1.56 x	82.77
Pyrethrin	1.24 ± 0.12	3.28	0.35	48.46 (38.21–58.80) ^{bcd}	–2.17 + 1.29 x	60.77
Nicotine	1.39 ± 0.12	1.09	0.78	122.16 (104.29–144.42)	–2.89 + 1.39 x	24.11
Osthole	1.56 ± 0.14	7.81	0.05	39.04 (31.60–46.37) ^{abc}	–2.86 + 1.78 x	75.44
Rotenone	1.12 ± 0.11	1.53	0.67	113.67 (93.72–139.19) ^{fg}	–2.30 + 1.12 x	25.91
<i>C. angulatus</i>	0.91 ± 0.13	0.06	0.10	738.48 (472.03–1542.41) ^{hi}	–2.63 + 0.92 x	3.99
Matrine	0.87 ± 0.12	0.21	0.98	369.95 (263.57–621.75) ^h	–2.24 + 0.87 x	7.96
Diflubenzuron	1.18 ± 0.12	0.36	0.95	61.45 (49.28–74.23) ^{cde}	–2.11 + 1.18x	47.93
Lufenuron	1.02 ± 0.11	0.02	0.10	111.38 (90.16–138.91) ^f	–2.09 + 1.02 x	26.44
Buprofezin	1.15 ± 0.13	4.10	0.17	29.45 (20.70–38.03) ^a	–1.84 + 1.24 x	100.00

SE, χ², df, CL and mg/L indicate standard error, chi-square, degrees of freedom, confidence limits and milligrams per liter, respectively. When the 95% confidence intervals for two or more LC₅₀ values for the same set of tested plants do not overlap (P 0.05), the results are statistically different.

Larval mortality at 24 h post-treatment was used to determine the toxicity regression equations, LC₅₀, and toxicity index. Emamectin benzoate, chlorantraniliprole, and chlorfenapyr were

shown to be more dangerous than indoxacarb, thiamethoxam, and imidacloprid in the studies presented. The LC₅₀ values for insecticides (emamectin benzoate and imidacloprid) ranged from

Table 4
The LT₅₀ values for selected botanical evaluated against 2nd instar larvae of *S. frugiperda*.

Botanicals	Concentrations (mg/L)	Fit of probe line Slope ± SE	χ ² (df = 3)	P value	LT ₅₀ (95% CL) (hours)	Reg. equation (y = a + bx)
Azadirachtin	25	4.17 ± 0.45	6.08	0.10	130.38 (119.26–147.60)	-7.34 + 3.4 x
	50	4.03 ± 0.37	6.24	0.10	103.82 (97.00–112.63)	-7.16 + 3.46 x
	100	3.68 ± 0.29	10.07	0.01	88.65 (72.96–116.09)	-6.54 + 3.35 x
	200	3.65 ± 0.26	5.66	0.12	68.85 (64.50–73.45)	-6.42 + 3.51 x
	400	3.88 ± 0.25	12.69	0.00	48.40 (36.19–59.95)	-6.72 + 4.02 x
Pyrethrin	25	3.92 ± 0.45	31.48	0.00	144.62 (136.45–160.20)	-6.5 + 2.87 x
	50	3.69 ± 0.36	30.64	0.00	123.77 (85.79–140.50)	-6.26 + 2.91 x
	100	3.45 ± 0.30	21.70	0.00	102.15 (74.97–253.36)	-6.06 + 2.99 x
	200	3.14 ± 0.25	13.75	0.00	82.03 (62.94–119.01)	-5.64 + 2.95 x
	400	3.27 ± 0.23	15.52	0.00	58.57 (41.64–77.33)	-5.77 + 3.29 x
Nicotine	25	2.69 ± 0.49	1.39	0.71	264.41 (194.72–493.97)	-5.79 + 2.32 x
	50	2.40 ± 0.36	1.57	0.67	230.31 (178.44–361.53)	-5.32 + 2.22 x
	100	2.63 ± 0.30	7.43	0.05	152.69 (131.64–189.81)	-5.17 + 2.33 x
	200	2.70 ± 0.26	7.57	0.05	110.20 (99.55–125.45)	-5.14 + 2.50 x
	400	2.94 ± 0.23	10.95	0.01	77.29 (60.20–105.81)	-5.30 + 2.81 x
Osthole	25	3.25 ± 0.36	2.26	0.52	138.06 (123.12–162.49)	-6.47 + 2.99 x
	50	3.22 ± 0.30	2.85	0.41	111.25 (101.89–124.34)	-6.22 + 3.02 x
	100	3.33 ± 0.27	6.39	0.09	88.68 (82.52–96.10)	-6.06 + 3.11 x
	200	3.64 ± 0.25	14.51	0.00	68.08 (52.06–88.25)	-6.30 + 3.45 x
	400	3.98 ± 0.25	15.48	0.00	48.91 (35.32–61.72)	-7.13 + 4.26 x
Rotenone	25	3.30 ± 0.52	0.79	0.85	200.68 (163.53–291.52)	-7.08 + 3.04 x
	50	2.91 ± 0.36	1.21	0.75	164.74 (140.98–20.9.41)	-5.97 + 2.66 x
	100	2.55 ± 0.28	0.83	0.84	139.07 (121.39–168.75)	-5.27 + 2.45 x
	200	2.65 ± 0.25	0.75	0.86	99.85 (90.82–112.14)	-5.18 + 2.59 x
	400	3.01 ± 0.23	1.27	0.74	70.03 (64.93–75.59)	-5.57 + 3.02 x
<i>C. angulatus</i>	25	3.19 ± 0.85	0.61	0.89	319.25 (208.68–1208.91)	-7.48 + 2.93 x
	50	2.34 ± 0.50	2.14	0.54	366.07 (236.21–1031.22)	-5.26 + 1.95 x
	100	2.14 ± 0.37	1.14	0.77	297.33 (210.33–586.20)	-5.00 + 1.98 x
	200	1.20 ± 0.29	2.34	0.50	229.75 (175.55–366.00)	-4.47 + 1.87 x
	400	1.94 ± 0.25	2.13	0.55	171.52 (139.88–236.49)	-4.18 + 1.86 x
Matrine	25	2.16 ± 0.45	0.63	0.89	385.72 (244.00–1122.95)	-5.26 + 1.98 x
	50	2.25 ± 0.36	1.48	0.69	258.14 (192.15–445.55)	-5.17 + 2.11 x
	100	2.09 ± 0.30	0.38	0.95	209.12 (164.44–313.10)	-4.77 + 2.05 x
	200	1.90 ± 0.25	0.31	0.96	161.22 (132.60–218.77)	-4.25 + 1.93 x
	400	1.75 ± 0.22	2.43	0.49	118.97 (101.63–149.30)	-3.75 + 1.81 x
Diflubenzuron	25	3.50 ± 0.47	3.31	0.35	169.57 (145.68–216.90)	-6.65 + 2.90 x
	50	3.42 ± 0.37	2.91	0.41	134.29 (120.75–155.85)	-6.51 + 3.02 x
	100	3.24 ± 0.29	3.80	0.28	105.69 (97.24–117.10)	6.09 + 3.00 x
	200	3.24 ± 0.26	3.58	0.31	82.53 (76.80–89.22)	-5.94 + 3.10 x
	400	3.04 ± 0.23	3.71	0.30	65.17 (60.39–70.24)	-5.40 + 2.99 x
Lufenuron	25	3.80 ± 0.58	0.73	0.87	181.31 (153.08–244.72)	-8.06 + 3.53 x
	50	3.06 ± 0.38	3.45	0.33	162.68 (140.15–204.51)	-6.02 + 2.67 x
	100	3.06 ± 0.31	0.58	0.90	124.21 (111.93–142.88)	-6.14 + 2.92 x
	200	2.91 ± 0.26	0.12	0.99	98.98 (90.78–109.87)	-5.74 + 2.88 x
	400	2.85 ± 0.24	0.39	0.94	78.10 (72.15–85.03)	-5.32 + 2.81 x
Buprofezin	25	4.04 ± 0.42	17.83	0.00	129.36 (97.93–753.96)	-6.84 + 3.15 x
	50	3.68 ± 0.35	16.45	0.00	115.58 (88.59–281.17)	-6.44 + 3.07 x
	100	3.82 ± 0.30	15.17	0.00	92.20 (72.82–135.82)	-6.64 + 3.37 x
	200	3.53 ± 0.26	15.38	0.00	75.38 (57.62–102.61)	-6.18 + 3.30 x
	400	3.55 ± 0.24	26.89	0.00	58.67 (35.96–84.90)	-6.31 + 3.61 x

SE, χ², df, CL and mg/L indicate standard error, chi-square, degrees of freedom, confidence limits and milligrams per liter, respectively.

0.319 to 13.556 mg/L, respectively, at 24 h post-treatment. The LC₅₀ values for chlorantranilprole and chlofenapyr ranged from 0.562 to 0.931 mg/L. They were generally lower than conventional insecticides (indoxacarb, thiamethoxam, and imidacloprid) with LC₅₀s ranging from 1.223 to 13.556 mg/L. The LC₅₀ values for insecticides (emamectin benzoate and imidacloprid) ranged from 0.785 to 55.229 mg/L, respectively, at 48 h post-treatment. The LC₅₀ values for chlorantranilprole ranged from 0.857 to 2.796 mg/L. They were generally lower than conventional insecticides (indoxacarb, thiamethoxam, and imidacloprid) with LC₅₀s ranging from 3.303 to 55.229 mg/L. The obtained results demonstrated that emamectin benzoate, chlorantranilprole were meaningfully more toxic to early instar *S. frugiperda* larvae than other tested insecticides. The LC₅₀ values for insecticides (emamectin benzoate and imidacloprid) ranged from 0.785 to 55.229 mg/L, respectively, at 72 h post-treatment. The LC₅₀ values of chlorantranilprole, ranged from

0.857 to 2.796 mg/L. They were generally lower than conventional insecticides (indoxacarb, thiamethoxam, and imidacloprid) with LC₅₀s ranging from 3.303 to 55.229 mg/L at 72 h post-treatment (Table 5).

Probit regression analysis revealed that emamectin benzoate and chlorfenapyr with maximum LT₅₀ values of 48.30 h (39.14–62.54), 53.19 (41.89–78.05), respectively, followed by chlorantranilprole 82.55 h (51.70–415.34) against 2nd instar *S. frugiperda* while other synthetic insecticides were exhibiting LT₅₀ values of 85.52 h (52.68–315.34) to 117.52 (75.54–715.34) treated with the lowest concentration of 3.125 mg/L.

At highest concentration of 50.0 mg/L, thiamethoxam and emamectin benzoate were proved to be effective exhibiting minimum LT₅₀ values of 9.57 h (0.73–17.69) and 53.19 h (41.89–78.05) and 10.18 h (4.48–14.57) respectively, followed by chlorantranilprole 10.57 (4.46–15.60) against 2nd instar *S. frugiperda* while other syn-

Table 5
LC₅₀ values for synthetic insecticides evaluated against 2nd instar larvae of *S. frugiperda* at different time intervals.

Fit of probe line							
Synthetic Insecticides	Time (Hours)	Slope ± SE	χ ² (df = 1)	P value	LC ₅₀ (95% CL) (mg/L)	Reg. equation (y = a + bx)	Toxicity index (%)
Emamectin benzoate	24	0.77 ± 0.11	0.04	0.10	0.32 (0.23–0.41) ^a	0.65 + 1.40 x	100.0
	48	1.18 ± 0.12	1.39	0.71	0.79 (0.63–0.956) ^a	0.13 + 1.20 x	100.0
	72	1.39 ± 0.15	7.15	0.07	0.26 (0.18–0.34) ^a	0.87 + 1.75 x	100.0
Indoxacarb	24	0.96 ± 0.11	1.42	0.70	2.39 (1.82–2.10) ^{fg}	–0.36 + 0.96 x	13.36
	48	0.78 ± 0.11	0.84	0.84	8.20 (6.09–12.511) ^{hij}	–0.71 + 0.78 x	9.57
	72	1.02 ± 0.12	1.97	0.58	1.81 (1.35–2.28) ^{bcd}	–0.27 + 1.04 x	14.51
Imidacloprid	24	1.01 ± 0.11	0.34	0.95	13.56 (10.8–16.80) ^k	–1.14 + 1.00 x	2.35
	48	0.10 ± 0.12	1.66	0.65	55.23 (40.90–85.55) ^l	–1.78 + 1.03 x	1.42
	72	1.05 ± 0.12	0.48	0.92	8.92 (6.92–11.01) ^m	–1.01 + 1.06 x	2.95
Thiamethoxam	24	0.71 ± 0.11	0.40	0.94	5.16 (3.09–7.22) ^j	–0.51 + 0.71 x	6.19
	48	0.58 ± 0.11	0.24	0.97	10.31 (6.72–14.91) ^{hijk}	–0.58 + 0.58 x	7.61
	72	0.70 ± 0.11	0.60	0.90	3.35 (1.69–5.02) ^l	–0.37 + 0.71 x	7.85
Chlorantraniliprole	24	1.06 ± 0.12	5.29	0.15	0.56 (0.42–0.71) ^b	0.27 + 1.10 x	56.76
	48	0.82 ± 0.11	2.38	0.50	0.86 (0.63–1.11) ^{ab}	0.06 + 0.82 x	91.60
	72	1.16 ± 0.13	2.71	0.00	0.39 (0.03–0.78) ^{ab}	0.50 + 1.33 x	66.92
Chlorfenapyr	24	0.82 ± 0.12	0.31	0.96	0.93 (0.52–1.34) ^{bcd}	0.03 + 0.82 x	34.26
	48	0.70 ± 0.11	0.26	0.97	3.30 (2.38–4.47) ^{cdef}	–0.37 + 0.70 x	23.77
	72	0.94 ± 0.12	0.33	0.96	0.72 (0.41–1.04) ^{bcd}	0.13 + 0.95 x	36.53

SE, χ², df and CL indicate standard error, chi-square, degrees of freedom and confidence limits, respectively. When the 95% confidence intervals for two or more LC₅₀ values for the same set of tested plants do not overlap (P 0.05), the results are statistically different.

Table 6
LT₅₀ values for synthetic insecticides evaluated against 2nd instar larvae of *S. frugiperda*.

Fit of probe line						
Synthetic Insecticides	Concentrations (mg/L)	Slope ± SE	χ ² (df = 1)	P value	LT ₅₀ (95% CL) (hours)	Reg. equation (y = a + bx)
Emamectin benzoate	3.125	1.33 ± 0.31	0.74	0.39	48.30 (39.14–62.54)	–2.24 + 1.33 x
	6.25	1.34 ± 0.31	0.41	0.52	29.26 (19.39–36.36)	–1.95 + 1.33 x
	12.5	1.32 ± 0.32	0.15	0.70	16.55 (6.85–23.44)	–1.59 + 1.31 x
	25.0	1.49 ± 0.35	0.36	0.55	10.43 (3.21–16.49)	–1.48 + 1.47 x
	50.0	2.80 ± 0.59	0.15	0.70	10.18 (4.48–14.57)	–6.72 + 4.02 x
Indoxacarb	3.125	0.92 ± 0.32	0.37	0.54	117.52 (75.54–715.34)	–2.00 + 0.97 x
	6.25	1.08 ± 0.31	0.17	0.68	71.27 (54.16–144.40)	–2.01 + 1.08 x
	12.5	1.31 ± 0.31	0.48	0.49	40.71 (31.62–50.54)	–2.11 + 1.31 x
	25.0	1.29 ± 0.31	0.65	0.42	25.15 (14.59–32.28)	–1.79 + 1.28 x
	50.0	1.72 ± 0.33	0.34	0.56	16.75 (9.40–22.25)	–2.08 + 1.70 x
Imidacloprid	3.125	1.93 ± 0.36	1.66	0.20	102.22 (80.20–164.31)	–4.00 + 2.00 x
	6.25	1.48 ± 0.32	0.30	0.59	82.74 (65.01–137.08)	–2.86 + 1.49 x
	12.5	1.54 ± 0.31	0.40	0.53	48.13 (40.20–59.45)	–2.60 + 1.54 x
	25.0	1.67 ± 0.31	0.63	0.43	32.90 (25.68–38.90)	–2.53 + 1.67 x
	50.0	2.01 ± 0.32	0.044	0.51	23.95 (17.73–28.73)	–2.74 + 1.99 x
Thiamethoxam	3.125	0.60 ± 0.30	0.05	0.83	85.52 (52.68–315.34)	–1.15 + 0.60 x
	6.25	0.66 ± 0.30	0.01	0.89	32.56 (2.36–49.54)	–1.00 + 0.66 x
	12.5	0.72 ± 0.30	0.01	0.98	21.51 (0.70–33.19)	–0.96 + 0.72 x
	25.0	0.73 ± 0.31	0.01	0.92	11.88 (0.02–22.75)	–0.78 + 0.73 x
	50.0	1.01 ± 0.33	0.07	0.80	9.57 (0.73–17.69)	–0.98 + 1.00 x
Chlorantraniliprole	3.125	0.61 ± 0.30	0.49	0.49	82.55 (51.70–415.34)	–1.17 + 0.61 x
	6.25	0.66 ± 0.30	0.07	0.79	35.12 (3.63–55.97)	–1.02 + 0.66 x
	12.5	0.78 ± 0.30	0.08	0.78	22.30 (2.37–33.25)	–1.05 + 0.78 x
	25.0	0.89 ± 0.32	0.00	0.94	10.46 (0.40–19.55)	–0.91 + 0.89 x
	50.0	1.98 ± 0.40	0.81	0.37	10.57 (4.46–15.60)	–2.19 + 2.08 x
Chlorfenapyr	3.125	1.14 ± 0.31	0.38	0.54	53.19 (41.89–78.05)	–1.96 + 1.14 x
	6.25	1.21 ± 0.31	0.27	0.61	32.11 (21.08–40.30)	–1.81 + 1.20 x
	12.5	1.19 ± 0.31	0.14	0.71	20.64 (9.07–28.21)	–1.55 + 1.18 x
	25.0	1.56 ± 0.33	0.22	0.64	15.77 (7.72–21.76)	–1.84 + 1.54 x
	50.0	1.72 ± 0.33	0.02	0.88	13.42 (6.35–18.93)	–1.93 + 1.71 x

SE, χ², df, CL and mg/L indicate standard error, chi-square, degrees of freedom, confidence limits and milligrams per liter, respectively.

tehtic insecticides were exhibiting LT₅₀ values of 13.42 h (6.35–18.93) to 23.95 h (17.73–28.73) (Table 6).

4. Discussion

The biorational insecticides have become an essential component for the development of integrated pest management

approach. Many researchers are working to manage the *S. frugiperda* in field and laboratory by various control tactics to develop the effective control practice as a persistent approach (Susanto et al., 2021b). Opting for botanical and synthetic insecticides would not only economical but also an environmental protection (Pavela, 2009). The use of botanical and synthetic insecticides against *S. frugiperda* can provide moderate efficacy levels with other control

measures, can lead to the effective management of *S. frugiperda* resulted in achieving better maize yields (Sisay et al., 2019).

The results of present study showed that mortality was concentration and time dependent against 2nd instar. Azadirachtin caused mortality of 45.30 and 96.70% at 24 and 120 h post-treatment, respectively. In line with the present study, azadirachtin was more effective in reducing the incidence in maize attacked by *S. frugiperda* larvae at one-week interval (Kammo et al., 2019). Azadirachtin and lufenuron showed highest larval mortality of 89.57 and 85.41%, respectively, against 6-day-old *S. frugiperda* (Tavares et al., 2010).

In the current research, pyrethrin induced 90.70% mortality in 2nd instar *S. frugiperda* at 120 h post-treatment. Pyrethrin shown very high efficiency against a variety of arthropod pests while having few negative effects on human health and the environment (Jeran et al., 2021). Pyrethroids have been reported as toxic against larvae and adult of *S. frugiperda* (Usmani and Knowles, 2001). The aqueous extract of tobacco leaf was applied by contact and residual assay gave larval mortality of 50.0 and 21.53% of *S. frugiperda* (Kardinan and Maris, 2021). The results further supported by (Sakadzo et al., 2020), where the extract of *N. tabacum* leaves toxic against early instar of *S. frugiperda*. The *Nicotiana tabacum* caused 62% larval mortality of *S. frugiperda* by feeding method (Phambala et al., 2020). Osthol is a coumarin compound play an essential role in plant defense responses (Chappell, 1995). Rotenone has a low level of insecticidal activity against tobacco cutworm, *Spodoptera litura* (Li et al., 2017).

Mythimna separata and *Agrotis ipsilon* are controlled by *Celastrus angulatus* (Cheng et al., 2016; Wang et al., 2017). Matrine inhibited the development of early third-instar oriental armyworm larvae, *Mythimna separata* Walker (Lepidoptera: Noctuidae) (Huang et al., 2017). It was shown that buprofezin was ineffective against *Helicoverpa armigera* at any of the doses studied. The populations of *H. armigera* were decreased and crop damage was much diminished when lufenuron was applied at both 37 and 49 g Al ha⁻¹ (Gogi et al., 2021).

In response to consumers' increasing concern about their health, regulations governing allowable amounts of pesticide residue on fruits and vegetables have tightened. However, pesticides have lasting effects and might be employed as emergency control of many arthropods, notably lepidopteran pests, after analyzing the optimal dosage level with the least residual effects. Insecticides examined demonstrated substantial effectiveness against *S. frugiperda* larvae in their second instar, as seen by the findings.

The findings of the current investigation are corroborated by Kulye et al. (2021) who found that emamectin benzoate and chlorantraniliprole are poisonous to *S. frugiperda*. Emamectin benzoate and chlorantraniliprole are more harmful to *S. frugiperda* early instar larvae (Deshmukh et al., 2020).

5. Conclusions

The effectiveness of the biological insecticides, including botanical and unconventional insecticides against *S. frugiperda* 2nd instar larvae after pest invasion in China have been evaluated in this study for the first time. Buprofezin, azadirachtin, osthole, and pyrethrin, four botanical insecticides, significantly killed second instar larvae of *S. frugiperda*. Moreover, these biorational pesticides may be included in an integrated pest management strategy for the long-term control of *S. frugiperda* in smallholder farmer settings.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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