



## Efficacy of Selected Pesticides against the Fall Armyworm Infestation in Small Holder Maize Production in Zambia

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### ABSTRACT

Fall Armyworm (FAW) has been a significant pest control issue since its invasion of Africa in 2016. Even though chemical pesticides pose risks to humans and the environment, they have been the most widely used short-term control method for FAW. A bio-efficacy study involving nine synthetic insecticides and one biopesticide was conducted in Zambia in 2020 and 2021 to evaluate their effectiveness against FAW and possible inclusion in an IPM strategy. The bioassay results revealed that all tested insecticides caused over 50% FAW larval mortality, with Acetamiprid, Imidacloprid, Emamectin benzoate+Lufenuron, and Chlorpyrifos+Cypermethrin being the most toxic. In the field trials, Emamectin benzoate+Lufenuron was the most effective insecticide, producing the least infestation (24.1%) and the highest grain yield (4817kg/ha), followed by Chlorpyrifos+Cypermethrin (26.4% and 4695kg/ha, respectively). Imidacloprid was the least effective, with a 32.5% infestation and a 4289kg/ha grain yield. On average, synthetic insecticides reduced FAW infestation, larval population, and leaf damage by more than 12–15% compared to the biopesticide Azadirachtin and 40–50% less infestation than the untreated control. Higher efficacy was also correlated with reduced grain damage, low cob rot infection, and higher grain yield in comparison with the untreated control. These insecticides could therefore be suitable components of an integrated pest management program for FAW in small-holder maize production, and three well-timed spray applications would successfully suppress the pest.

**Keywords:** Fall Armyworm, Infestation, Maize, Insecticides, Integrated pest Management

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### INTRODUCTION

Fall armyworm, *Spodoptera frugiperda* (FAW), is a highly destructive insect pest that first appeared in Africa in January 2016 and has since spread to all sub-Saharan African (SSA) countries except Lesotho (Rwomushana et al., 2018; Matova et al., 2020; Timilsena et al., 2022). FAW infests and feeds on maize, sorghum, and other SSA crop species and their wild relatives, causing devastating impacts on agriculture (Guimapi et al., 2022; Aleem et al., 2023). The moths' ability to fly up to 100km, their diverse host range, and their lack of diapause growth phase make it difficult to control, manage, or eradicate FAW (Anjorin et al., 2022). Since its arrival, 11–54% of maize production has

been lost annually (Abro et al., 2021; De Groote et al., 2020; Overton et al., 2021).

The FAW has been recognized as a devastating pest since its invasion in Zambia, where it has spread to all ten provinces (Kabwe et al., 2018; Kasoma et al., 2021; Durocher-Granger et al., 2021) and caused farm-level yield losses of more than 35% (Rwomushana et al., 2018). But in some areas, FAW is capable of causing a 100% yield loss due to its unforeseen occurrence from the seedling to the cob formation stage (Kumar et al., 2022). As a result, synthetic chemical insecticides are widely used as a main method of control (Harrison et al., 2019; Kasoma et al., 2021; Kalyebi et al., 2023), although their efficacy has not been proven. In addition, newer insecticides like diamides,

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ivermectin, spinosyns, and benzylureas have yet to be exposed to the fall armyworm. However, several studies (Rwomushana et al., 2018; Kansime et al., 2019; Chimweta et al., 2020; Tambo et al., 2020) have reported that the most common pesticides used by farmers in controlling the FAW are Cypermethrin, Lambda-cyhalothrin, Chlorpyrifos, Emamectin benzoate, Imidacloprid, Lindane, Acetamiprid, Spinetoram, Deltamethrin, Permethrin, Maltodextrin, Ethyl palmitate, Carbaryl, Malathion, and Fipronil.

In Zambia, the government spends between USD 2.5 and 5 million annually to procure and distribute synthetic insecticides to small-scale farmers (Ministry of Agriculture, 2018-2022; Kabwe et al., 2018). Despite the risks associated with the potential contamination of the environment and the negative impact on human and animal health, pesticides are still crucial for increased crop production and insect pest management (Lykogianni et al., 2021). Several reports also indicate that effective control of FAW could still be achieved through the use of synthetic insecticides (Sileshi et al., 2022; Soyel et al., 2023), and chemical control of FAW armyworm is still the most popular method of control (Kansime et al., 2019).

Although heavy infestations of fall armyworm may have justified chemical control, their frequent applications are unsustainable as they may lead to the development of insecticide resistance, increase production costs, and cause a decline in agrobiodiversity as well as health risks to growers and consumers (Ahissou et al., 2021; Safdar et al., 2022). Moreover, smallholder farmers with limited resources cannot afford expensive chemical insecticides for the control of Fall armyworm and are often unwilling or unable to purchase appropriate chemicals and safety equipment. Some farmers have resorted to using highly dangerous chemicals to ward off the fall armyworm, and, in some cases, using lower than the recommended dosage rates or carrying out prophylactic sprays of pesticides can cause undesirable changes in the pest's gene pool, potentially lead to insect resistance (Chaud et al., 2021; Otim et al., 2021; Van den Berg and Du Plessis, 2022).

Furthermore, several insecticide molecules have been developed and made available on the pesticide market. It is therefore essential to identify effective pesticides that can be recommended for use by extension services and

farmers as part of an integrated pest management strategy against FAW. In the current study, a few selected synthetic insecticides were evaluated for their effectiveness against the fall armyworm under laboratory and small-holder farmer field conditions and generated bio-efficacy data that can be used to integrate synthetic pesticides with other integrated pest management practices.

## MATERIALS & METHODS

### Laboratory Bioassay

A laboratory trial was conducted in the Entomology Laboratory at the Mount Makulu Central Research Station (MMRC), Chilanga, to evaluate nine (9) synthetic insecticides and one biopesticide in January 2020. The synthetic insecticides were Regent (Fipronil), Assail (Acetamiprid), Cyclone (Chlorpyrifos+Cypermethrin), Tracer 480 (Spinosad), Belt (Flubendiamide), Advise 2 FL (Imidacloprid), Denim Fit (Emamectin benzoate+Lufenuron), Malathion 50% EC (Malathion 500 g/L), and Orthene (Acephate) (Table 1). The biopesticide was a neem-derived biopesticide, Nimbecidine (Azadirachtin and other limonoids). All the synthetic insecticides and the biopesticide were sourced locally from Farmers Barn, Syngenta, Osho Chemicals, and Crop Serve (Zambia) Limited in Lusaka. All insecticides tested were either moderately or slightly hazardous, implying that they have a relatively low level of toxicity to both humans and animals (WHO, 2019). Before application, each insecticide was thoroughly mixed with water following the manufacturers' recommendations for 5–10 min.

The 3<sup>rd</sup> instar larvae, derived from a field population of FAW collected from unsprayed Mount Makulu maize fields, were used in the experiment. The larvae were maintained in the Entomology Laboratory, Mount Makulu Central Research Station, at 23.9 to 29.4 °C, 80% relative humidity, and a 12:12 h (L:D) photoperiod.

Freshly harvested leaves from 20-day-old maize seedlings, Seedco hybrid 'SC 513', were thoroughly cleaned with under-tap water, then sterile water, and dried using paper towels. Thereafter, the leaves were chopped into uniform-sized leaf bits (3cm length×1cm width) and placed on damp Whatman No. 10 filter paper that lined

**Table 1:** Trade name, active ingredient, family class, mode of action, manufacturer and rate of insecticides

Pesticide (Trade Name)	Active Ingredient	Family class	Mode of action	Manufacturer	Recommended dose/rate
Regent	Fipronil	Phenylpyrazole	Contact	Bayer Crop Science	0.5g/L water
Assail	Acetamiprid	Neonicotinoid	Broad Spectrum	UPL	25mL
Cyclone	Chlorpyrifos +Cypermethrin 5% EC	50% Organophosphate/pyrethroid	Dual knockdown, stomach posing	Fil Industries, India	2mL/L water
Tracer 480	Spinosad	Spinosyn	Contact, Ingestion	Corteva Agriscience	150mL/ha
Belt	Flubendiamide	Phthalic acid diamide	Stomach action and muscular disruption	Crop Bayer	120mL/ha
Nimbecidine	Azadirachtin and other limonoids	Azadirachtin	Antifeedant and repellent	Osho Chemicals	3 litres/ha
Advise 2 FL	Imidacloprid	Neonicotinoids	Contact, stomach toxicity and internal inhalation	Bayer Crop Science	112.5mL/ha
Denim Fit	Emamectin benzoate+Lufenuron	Avermectin/n-phenylureas	Translaminar (stomach poison)	Syngenta	0.35g/L water
Malathion EC	50% Malathion 500 g L <sup>-1</sup>	Organophosphates.	Contact, inhalation, ingestion	Drexel chemicals	2L/ha
Orthene	Acephate	Organophosphate	Ingestion, Contact/Systemic	United Phosphorus limited	0.5g/L water

the bottom of the 150×15mm polystyrene petri dishes (Fischer Scientific, United States). The filter paper was moistened with a test insecticide solution. The same insecticide solution was sprayed on the leaf fragments for 10 to 20 seconds using a hand sprayer.

After which, ten (10) fall armyworm larvae were introduced into each petri dish and exposed to treated leaf bits for a period of 72h. Each petri dish has a perforated cover lid with spaced slits not measuring more than 0.5cm each. The vapors from the treated leaves mimicked the field spray coverage. The controls were Petri dishes with leaf bits and filter paper treated with sterile water. Each treatment was replicated five times in a completely random design and repeated three times on different days. A total of 1650 larvae were used for the bioassay. The numbers of live and dead larvae were recorded before spray and at 12, 24, 36, 48 and 72h after application. Dead larvae were confirmed by a lack of movement after being probed for 1 minute, and the percent mortality rate of larvae was calculated and corrected according to the formula of Abbot (1925):

$$\text{Percent mortality} = (Lt - Li)/Lt \times 100;$$

where Lt is the total number of larvae per treatment and Li is the number of live larvae after exposure.

## Field Studies

### Sites

Four (4) best-performing insecticides and 1 biopesticide from the Laboratory bioassay were evaluated in field trials at three sites: Ebenezer Child Trust Farm (ECTF), Livingstone (17° 51' 11.292" S, 25° 53.323" E, 917m above sea level, agro-ecological region (AER I), and Mount Makulu Central Research Station, Chilanga (15° 33'S, 28° 15'E, 1213m above sea level, AER II) during the 2020 and 2021 rainy seasons; Sons of Thunder Farm (SoT), Livingstone (17° 39' 9.961 S, 25° 56.334" E, 917m above sea level), AER I in 2020; and Copperbelt Research Station Field (CRS), Lukoshi Ward, Kalulushi (12° 50' 29.4" S, 28° 853.667" E, 1208m above sea level), AER III, in 2021 (Fig. 1). AER I is characterized by a mean annual rainfall of less than 800 mm, a relatively short crop growing season of 80–120 days, and poorly distributed rains, which often result in crop failure due to persistent dry spells and droughts. It also experiences very high temperatures (35–40°C) between September and November.

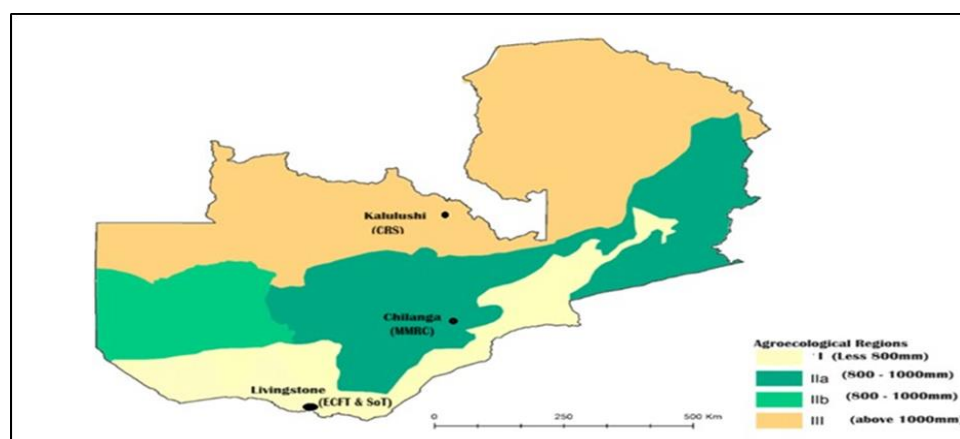
AER II receives between 800 and 1000mm of rainfall annually, which is evenly distributed throughout the crop-growing season. The growing season is slightly longer than in AER I, between 100 and 140 days. The average annual temperature during the crop-growing season (November to April) is between 22 and 29 °C. On the other hand, AER III is characterized by a mean annual rainfall of more than 1000 mm, a relatively long crop-growing season (greater than 120 days), fairly well-distributed rains, and high humidity between January and March. The sites for the field trials were purposefully selected based on the previous government reports of FAW infestation (Ministry of Agriculture, 2018–2022) and the ideal environment for the growth of the target crop.

Mount Makulu Central Research Station is located 15km south of Lusaka, the capital city of Zambia. Ebenezer Child Trust Farm and Sons of Thunder Farm are 514 and 457km, respectively, south of Lusaka, and Copperbelt Research Station in Kalulushi is 435km north of Lusaka. The soils at ECTF and SoT are primarily Arenalsols, sandy to clay loam, while those at MMRC are mainly acrisols (World Reference Base for Soil Resources /FAO Classification) loamy sand or sandy loam. Soils at CRS are typic acrisols and Leptosols (World Reference Base for Soil Resources /FAO Classification). If properly maintained, any of these soils could yield a healthy crop of maize.

### Treatments

Four (4) best-performing chemical insecticides from the bioassay, namely Assail (Acetamiprid), Cyclone (Chlorpyrifos+Cypermethrin), Advise 2 FL (Imidacloprid), and Denim Fit (Emamectin benzoate+Lufenuron), one (1) biopesticide neem-derived biopesticide, Nimbecidine (Azadirachtin), and the control (untreated maize), were evaluated in a randomized complete block design (RCBD) with 4 replications at each site. The inclusion of Azadirachtin was to facilitate a comparison of the effects of synthetics and biopesticides in general and to act as a positive control. Untreated maize was the negative control.

Early maturing white dent-grained maize hybrid Seed Co. Maize variety, SC 513 seeds with a yield potential of up to 10 t ha<sup>-1</sup> were sown at 2–3cm depth, at 25cm and 75cm, intra-row and inter-row spacing, respectively. The maize seed was sourced from Seed Co. (Zambia Limited), Lusaka. Each experimental plot, 6x6m (36 m<sup>2</sup>), had 216 plants (9



**Fig. 1:** Map of Zambia showing the sites where the field experiment was set up.

rows) and was sprayed with a single designated test material, except the no-spray (untreated) control. A 1.5-meter alley or buffer zone was included between experimental plots and replications to minimize insecticide drift. Standard agricultural practices were followed. Basal fertilizer (NPK 10-20-10) at the rate of 200kg/ha at planting and top-dressing fertilizer Urea (46% N) at the same rate. Weeding was done using a hand hoe and carried out thrice: at 3, 5, and 7 weeks old.

The chemical insecticides were applied three (3) times, at 14, 24, and 35 days after crop emergence, using a 20-liter Jacto knapsack sprayer. Before spraying, each of the selected pesticides was thoroughly mixed with water following the manufacturers' recommendations for 2–5 minutes. After each treatment, the knapsack sprayer tank was emptied of any remaining insecticide, then rinsed with liquid soap. The tank was then filled with warm water and left to stand for a few minutes. It was thereafter shaken to loosen any residue, then emptied. The wand, nozzle, shut-off valve, and other components were then rinsed with clean water. Data were collected 2 days before the first spray, i.e., 12 days after crop emergence (DAE), and 6–7 days after the first, second, and third sprays (20, 30, and 42 DAE). Data were collected on the number of plants infested, larvae per plant (i.e., both dead and live), and leaf injury. The levels of insect injury were rated using a modified scale of 1–9 described by Ni et al. (2011) based on the rating scale of Davis et al. (1992), where 1 = no injury and 9 = most leaves with long lesions and complete defoliation. Leaf damage was scored for the top three leaves and the whorl only to avoid counting damage from previous assessments (Chisonga et al., 2023).

At harvest, the percentage of cobs with characteristic signs of FAW damage was assessed on 20 randomly selected cobs per plot using the rating scale proposed by Prasanna et al. (2018) of 1–9, where 1 = no grain damaged and 9 = 75% damaged. Maize ear rot was assessed using a seven-point scale: 1 = no visible disease symptoms, 2 = 1–3%, 3 = 4–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–75%, and 7 = > 75% of kernels exhibiting visual symptoms of infection, such as brown, pink, or reddish discoloration of kernels and pinkish or complete rotten with white mycelial growth (Czembor et al., 2019; Talba et al., 2023).

Marketable grain yield (kg/ha) was assessed per plot by collecting all grains from the four inner rows (neglecting the outer two from the total of six rows per plot) and pooling and expressing them in tons per ha. The grain yield per hectare was obtained by multiplying the field weight, i.e., grain yield per plot, divided by the plot area, after adjusting for a moisture content of 12–13%, i.e.,

$$GY \text{ (kg/ha)} = [\text{Grain Weight} \times (100 - \text{MC}) / (100 - \text{Adjusted MC})] * [10000 / \text{Plot Area}]$$

where grain weight is in kg, moisture content (MC) is in percentage (%), and plot area is in m<sup>2</sup>.

### Statistical Analysis

Data from both the bioassay and field experiments were subjected to the analysis of variance (ANOVA), and the means were compared by Tukey's honest significant

difference test and Fisher's least difference test at 5% probability after having tested the normality and homoscedasticity of the data through Shapiro-Wilk and Bartlett tests, respectively. Percent larval mortality from laboratory bioassays and percent FAW plant infestation, cob damage, and the incidence and severity of cob rot from field trials were transformed using an arcsine, while the leaf injury score and number of larvae per plant were transformed to  $(x + 0.5)^{1/2}$  was applied to stabilize the variance; however, untransformed data are presented in the tables. All statistical analyses were performed using the GENSTAT® statistical program, 18th Edition (VSN, 2015).

## RESULTS

### Laboratory Study of Insecticide against Fall Army Worm

At each exposure time, there was a significant difference ( $P < 0.05$ ) in the effect of the insecticides on the percent FAW larvae mortalities in comparison to the untreated control (Table 2). No larval mortality was recorded in untreated control throughout the duration of the study. At 12h post-treatment ( $F_{10,132} = 315.4$ ;  $P < 0.001$ ), the larval mortality ranged from 8.8 to 38.4%; the highest mortality was in larvae treated with Acetamiprid, which had the highest mortality (38.4%), followed by Emamectin benzoate+Lufenuron (33.07%), and the lowest was in those treated with Flubendiamide (8.8%).

At 24h post-treatment ( $F_{10,132} = 495.7$ ;  $P < 0.001$ ), a significant but gradual increase in mortality was observed in all the insecticide-treated larvae; larval mortality ranged from 7.2 to 23.3%. The highest mortality was in the Chlorpyrifos+Cypermethrin-treated larvae (50.9%), followed by Acetamiprid at 45.6%, and the lowest was in the Acephate treatment at 17.3%.

At 36h post-treatment ( $F_{10,132} = 659.8$ ;  $P < 0.001$ ), the highest mortality was in the FAW larvae treated with Emamectin benzoate+Lufenuron (60.0%), followed by those treated with Acetamiprid (57.6%). The lowest mortality was in Acephate treated larvae (26.9%). On average, at this observational time, FAW larvae mortality increased significantly by more than 10.2% across all treatments.

At 48h post-treatment ( $F_{10,132} = 934.9$ ;  $P < 0.001$ ), the highest mortality was in the Chlorpyrifos+Cypermethrin-treated larvae (72.8%), followed by Emamectin benzoate+Lufenuron (71.5%), and the lowest was Acephate (38.9%). At 72h post-treatment, all the synthetic insecticides, including the biopesticide Azadirachtin, caused significantly high larval mortality ( $F_{10,132} = 1218.7$ ;  $P < 0.001$ ). The larval mortality ranged between 50.9 and 86.9 (Table 2). The highest FAW larvae mortality was in the Acetamiprid treated maize (86.9%), which was followed by Chlorpyrifos+Cypermethrin (81.8%). Acephate treatment resulted in the lowest mortality (50.9%) among the insecticides tested, making it the least effective. The biopesticide Azadirachtin produced 58.9% mortality. In terms of the order of performance: Acetamiprid > Chlorpyrifos+Cypermethrin > Emamectin benzoate+

**Table 2:** Mean percent mortality of the FAW larvae after 72-hr post-insecticide treatment

Treatment	Percent larval mortality				
	12-hr	24-hr	36-hr	48-hr	72-hr
Imidacloprid	29.3ef	41.3d	53.3ef	69.6f	77.3e
Acetamiprid	38.4h	45.6e	57.6gh	65.9e	86.9g
Flubendiamide	8.8b	19.2b	31.2c	43.2c	55.2c
Chlorpyrifos +Cypermethrin	31.7e	50.9f	55.7fg	72.8f	81.9f
Emamectin benzoate+Lufenuron	33.1g	40.8d	60.0h	71.5f	81.6f
Malathion	13.1cd	25.1c	32.8cd	41.6bc	53.6bc
Azadirachtin	13.9d	24.5c	35.7d	47.2d	58.9d
Acephate	10.1bc	17.3b	26.9b	38.9b	50.9b
Fipronil	14.1d	23.5c	35.5d	47.5d	59.5d
Spinosad	27.2e	44.3de	51.2e	63.2e	75.2e
Control	0.0a	0.0a	0.0a	0a	0a
Mean	19.8	30.2	39.2	51	61.9
DF	10	10	10	10	10
F value	315.3	495.7	659.8	934.9	1218.7
Prob	<0.001	<0.001	<0.001	<0.001	<0.001

NB. Within columns, figures followed by the same letters are not significantly different ( $P < 0.05$ ; Tukey's HSD test).

Lufenuron > Imidacloprid > Spinosad > Fipronil > Azadirachtin > Flubendiamide > Malathion > Acephate.

## Field Experiments

### Effect on Insecticides on FAW Infestation

There were significant differences for the effects of insecticides ( $F_{5,332} = 138.42$ ;  $P < 0.001$ ), number of sprays ( $F_{2,332} = 6.55$ ;  $P < 0.002$ ), and insecticide x number of sprays interaction ( $F_{10,332} = 4.95$ ;  $P < 0.001$ ). A significantly lower number of plants ( $P < 0.001$ ) were infested by FAW in the insecticide-treated plots compared to the control, which had on average 74.8% infestation (Table 3). After the first spray, the lowest FAW infestation was in Chlorpyrifos+Cypermethrin (30.9%), and the highest was in Azadirachtin-treated maize (39.5%). After 2<sup>nd</sup> spray, the reduction in FAW infestation ranged from 1.9–9%, with the biggest reduction occurring in the maize treated with Emamectin benzoate+Lufenuron and the least in Azadirachtin-treated crops. When the third spray was applied, the reduction ranged from 4 to 10.7%. The biggest reduction was in Chlorpyrifos+Cypermethrin, followed by Acetamiprid treated maize, and the least was in Emamectin benzoate+Lufenuron. On average, after the three sprays, the lowest infestation was in Emamectin benzoate+Lufenuron-treated maize (24.6%), followed by Chlorpyrifos+Cypermethrin (26.1%), and the highest was in maize treated with Imidacloprid (32.5%) (Table 3). The insecticide treatment exerted a 9.2 to 13.9% reduction in FAW infestation between the first and third sprays. This indicated an average reduction of 52.4–68% in FAW infestation compared to the untreated control. In terms of performance, the rank order of insecticides is: Emamectin benzoate + Lufenuron > Chlorpyrifos + Cypermethrin > Acetamiprid > Imidacloprid > Azadirachtin > untreated control.

There was also significant season x site x pesticide interaction ( $F_{5,332} = 2.69$ ;  $P < 0.001$ ) was also observed (Fig. 2). There was a significantly higher FAW infestation in 2020 than in 2021. The highest FAW infestation was recorded at MMCR in 2020 (44.1%), followed by ECTF (34.2%), the same year. The lowest was at CRS in 2021 (17.2%). MMCR may have had high FAW infestation probably as a result of continuous cropping of the FAW preferred host, maize, unlike in the other sites. Among insecticides, the lowest

infestation occurred when Emamectin benzoate +Lufenuron were applied in 2021 at CRS (8.3%) and the highest was in the maize treated with Azadirachtin at Mount Makulu in 2021(44.6%).

### Effect on Insecticides on FAW Leaf Injury

There were significant treatment effects ( $F_{5,332} = 379.01$ ;  $P < 0.001$ ) for the FAW leaf injury. The highest FAW leaf damage was in the untreated control (3.46), followed by maize treated with Azadirachtin (1.85) (Table 4). The lowest was in Emamectin benzoate+Lufenuron (1.54). The leaf injury in the chemical insecticide-treated maize plants was significantly lower ( $P < 0.05$ ) compared to both the untreated control and Azadirachtin-treated crop. Among the chemical insecticides, the lowest FAW injury was in the maize treated with Emamectin benzoate + Lufenuron, followed by Chlorpyrifos+Cypermethrin (Table 4). The highest leaf injury was in Imidacloprid-treated maize (1.67).

There were also significant spray time x treatment interaction effects ( $F_{10,332} = 29.45$ ;  $P < 0.001$ ) After the first spray of the insecticides, the injury score in the treated plants was between 24 and 38% lower than in the untreated maize (Table 4). When the second spray was made, the overall injury score was reduced by an additional 6–10%, and after the third spray, by 5–15%. When the third spray was applied, the reduction in the overall FAW leaf injury score was 52–56% lower in the chemical insecticide and 47% less in Azadirachtin than in the untreated control. Throughout the duration of the field trials, the untreated control recorded a significant increase in leaf injury of more than 123% due to an increase in Fall armyworm larvae feeding on fresh leaves.

Season x site x treatment interaction effects ( $F_{5,332} = 3.33$ ;  $P < 0.006$ ). Significantly more leaf damage was recorded in 2020 compared to 2021 (Fig. 3). In 2020, the lowest FAW leaf damage was in Chlorpyrifos +Cypermethrin treated maize at SoT, which had the lowest leaf damage (1.73). The highest was in Acetamiprid treated maize at MMCR (2.03) (Fig. 3). In 2021, the lowest FAW leaf damage was in Emamectin benzoate+Lufenuron-treated maize at CRS (1.13), and the highest was in Imidacloprid-treated maize at MMCR (1.52). It was nevertheless observed that maize treated with synthetic insecticides had

**Table 3:** Percentage of plants with FAW infested plants (FAW larvae symptoms on upper leaves and whorl) at three different spraying times

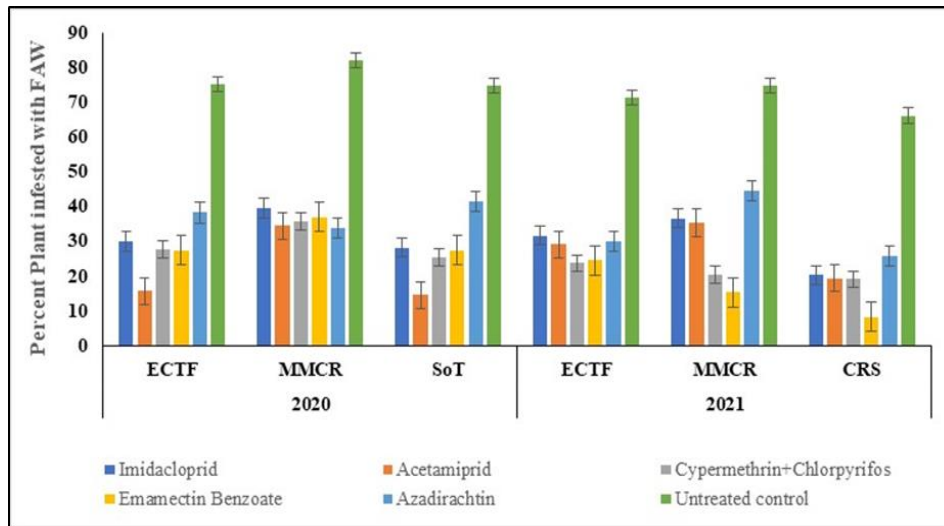
Treatments	Number of Insecticide Sprays					%Above Untreated Control
	Pre-Spray	1 <sup>st</sup> Spray	2 <sup>nd</sup> Spray	3 <sup>rd</sup> Spray	Average*	
Imidacloprid	37.4b	38.2ab	33.3ab	26.4ab	32.5bc	-57
Acetamiprid	31.3a	32.1ab	29.3ab	20.0a	26.6ab	-64
Chlorpyrifos+Cypermethrin	31.6a	30.9a	29.0ab	18.4a	26.1ab	-65
Emamectin benzoate+Lufenuron	30.4a	32.3ab	23.3a	18.4a	24.6a	-67
Azadirachtin	32.9ab	39.5b	37.0b	31.9b	36.1c	-52
Control	36.0ab	63.8c	77.6c	82.8c	74.8d	
Mean	33.3	39.5	38.3	33.0	36.8	
DF	5	5	5	5	5	
F-value	1.77	22.5	46.5	85.8	138.4	
Prob	0.125	<0.001	<0.001	<0.001	<0.001	

NB. Figures within a column followed by same letter are not significant different at Fisher's LSD test; P<0.05; DF=Degrees of Freedom; Prob=P-value. \*Average effect of 1st 2nd and 3rd Sprays

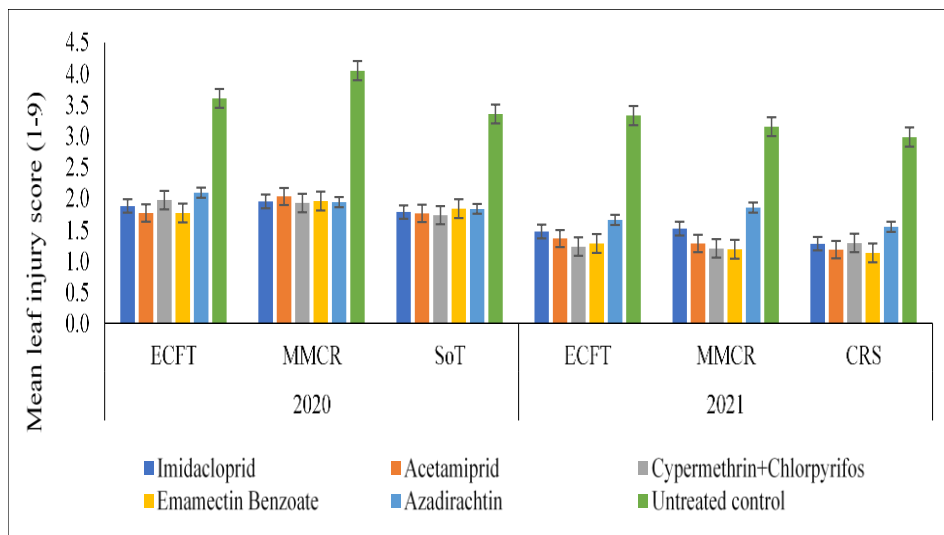
**Table 4:** Mean leaf injury score (1-9) at three different spraying times

Treatments	Number of Insecticide Applications					% Above Untreated Control
	Pre-spray	1 <sup>st</sup> Spray	2 <sup>nd</sup> Spray	3 <sup>rd</sup> Spray	Average	
Imidacloprid	1.63ab	1.85ab	1.68a	1.48ab	1.67a	-52
Acetamiprid	1.62ab	1.75a	1.64a	1.44ab	1.60a	-54
Chlorpyrifos+Cypermethrin	1.55a	1.71a	1.58a	1.43ab	1.57a	-55
Emamectin benzoate+Lufenuron	1.59a	1.70a	1.56a	1.36a	1.54a	-56
Azadirachtin	1.76b	2.05b	1.90b	1.61b	1.85b	-47
Control	1.69ab	2.89c	3.30c	4.20c	3.46c	
Mean	1.64	1.99	1.94	1.92	1.95	
DF	5	5	5	5	5	
F-value	2.12	49.04	124.82	257.89	379.01	
Prob	0.069	<0.001	<0.001	<0.001	<0.001	

NB. Figures within a column followed by same letter are not significant different at Fisher's LSD test; P<0.05; \*Average of treatment effect i.e., 1st, 2nd and 3rd Spray.



**Fig. 2:** Mean percent plant infested by Fall armyworm in the insecticide treated maize at different sites in 2020 and 2021.



**Fig. 3:** Mean leaf FAW injury score in insecticide treated maize at different sites in 2020 and 2021.

**Table 5:** The number of larvae per plant at three different spraying times

Treatment	Number of insecticide applications				Average*	% above Control
	Pre-spray	1 <sup>st</sup> Spray	2 <sup>nd</sup> Spray	3 <sup>rd</sup> Spray		
Imidacloprid	0.82b	0.86ab	0.42a	0.25a	0.51ab	-66
Acetamiprid	0.74ab	0.82ab	0.44a	0.29a	0.52ab	-65
Chlorpyrifos + Cypermethrin	0.61a	0.65a	0.46ab	0.25a	0.45ab	-70
Emamectin benzoate+Lufenuron	0.66a	0.69ab	0.37a	0.22a	0.42a	-72
Azadirachtin	0.73ab	0.77ab	0.62b	0.47b	0.62b	-59
Control	0.72ab	1.11b	1.31c	2.06c	1.50c	
Mean	0.71	0.81	0.61	0.58	0.67	
DF	5	5	5	5	5	
F-value	1.83	3.58	38.65	216.82	84.59	
Prob	<0.134	<0.005	<0.001	<0.001	<0.001	

NB. Figures within a column followed by same letter are not significant different at Fisher's LSD test; P<0.05; \*Average effect of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> Spray.

4.6 and 23.9% FAW leaf damage compared to Azadirachtin-treated maize in the 2020 and 2021 seasons, respectively. On the other hand, the synthetics were 5.8–12.9% more effective than Azadirachtin in reducing FAW leaf damage.

### Effect of Insecticides on FAW Larval Population

There were significant treatments ( $F_{5,332}=99.45$ ;  $P<0.001$ ) for the FAW Larval population. There were significantly more larvae in untreated control compared to insecticide treatments (Table 5). Among the insecticides, the lowest larvae/plant were found in the maize treated with Emamectin Benzoate + Lufenuron (1.54/plant), followed by Chlorpyrifos + Cypermethrin (1.57). The highest was in the maize treated with imidacloprid (1.67 per plant). The insecticide treatments had 66–72% fewer FAW larvae than the untreated control and 6–13% less than those recorded in the Azadirachtin treatment compared to the insecticide treatments (Table 5). The superior performance of synthetics compared to biopesticides may be due in part to the quick kill effect on FAW larvae compared to Azadirachtin.

Significant differences in FAW numbers were observed 6–7 days after the first ( $F_{5,106}=3.58$ ;  $p<0.005$ ), second ( $F_{5,106}=38.65$ ;  $P<0.001$ ), and third ( $F_{5,106}=216.82$ ;  $P<0.001$ ) applications. On day 6 after the first application, the lowest mean values were observed, with the lowest number being plants in Chlorpyrifos+Cypermethrin-treated maize, followed by those sprayed with Emamectin Benzoate, and no significant differences were found between Emamectin benzoate+Lufenuron with other insecticides and also with untreated control. Six days after the second application, the insecticide treatment differed significantly with the lowest mean values obtained with Emamectin benzoate+Lufenuron, and significantly lower FAW numbers were found in the insecticide-treated plots as compared to the untreated control. At the last sampling date, 6 days after the third application, the lowest FAW population was in Emamectin Benzoate + Lufenuron (0.22/plant), whereas the highest was in untreated maize (2.06/plant) (Table 5). There were no significant differences

observed among the synthetic insecticide treatments, but they differed significantly from the FAW numbers found in Azadirachtin-treated maize and untreated maize. Two times more FAW numbers were recorded in the Azadirachtin plots compared to synthetic insecticide-treated maize. Overall, Imidacloprid caused the biggest reduction in FAW larva population after the three sprays, 69.1% lower than its pre-spray levels, followed by Emamectin benzoate + Lufenuron (66%). This may imply that foliar application of the insecticides could have suppressed FAW population build-up, thereby reducing any further leaf damage.

### Response of FAW Infestation to Number of Foliar Applications of Insecticides

The results in Table 6 show that a significant reduction in FAW infestation with an increase in the number of sprays of Acetamiprid, Chlorpyrifos, Emamectin benzoate+Lufenuron, and Azadirachtin, with the biggest reduction occurring in Acetamiprid-treated maize, followed by Chlorpyrifos + Cypermethrin and Emamectin benzoate+Lufenuron-treated maize. Further, the results of the regression analysis indicated no significant ( $P<0.007$ ) reduction in FAW infestation that could be obtained from an additional spray of Imidacloprid compared to the three other insecticides and biopesticide.

### Effect of Insecticides on Cob Damage, Cob Rot and Grain Yield (kg/ha)

The treatment differed significantly for the degree of FAW cob damage ( $F_{5,105}=22.55$ ;  $P<0.001$ ), incidence ( $F_{5,105}=31.48$ ;  $P<0.001$ ) and severity of cob rot ( $F_{5,105}=18.9$ ;  $P<0.001$ ), and grain yield ( $F_{5,105}=49.37$ ;  $P<0.001$ ) (Table 7). No significant environmental effects were observed for these parameters. When compared to the untreated control, the Emamectin Benzoate+Lufenuron application resulted in a significant reduction in cob damage (-0.94), cob rot incidence (-50.2%), and cob rot severity (-73.4%), followed by Chlorpyrifos+ Cypermethrin-treated maize, which had similar pattern of results. Insecticide treatments did not, however, significantly differ in terms of cob damage

**Table 6:** Response of FAW infestation after three sprays of selected insecticides

Treatment	Slope ± SE	Intercept + SE	F value	F pr.
Imidacloprid	-4.27±2.38	39.6±5.15	3.21	0.077
Acetamiprid	-6.04±2.42	37.5±5.22	6.25	0.015
Chlorpyrifos+Cypermethrin	-5.73±2.52	36.9±5.44	5.17	0.026
Emamectin benzoate+Lufenuron	-5.73±2.35	34.9±5.07	5.96	0.017
Azadirachtin	-5.10±2.14	44.4±3.76	6.31	0.014
Untreated control	7.41±2.33	61.0±4.98	18.85	<0.001

N.B \* Significant at  $P<0.05$

**Table 7:** Effects of different insecticide treatments on percent cob damage, cob rot and grain yield (kg/ha)

Treatment	Percent cob rot		Cob damage	Grain yield	% Above control – grain yield
	Incidence <sup>†</sup>	Severity <sup>†</sup>	(%)	kg/ha	
Imidacloprid	44.9ab	8.0c	1.5a	4289c	52.4
Acetamiprid	52.6bc	6.9bc	2.3a	4445cd	57.9
Chlorpyrifos + Cypermethrin	37.8a	4.9ab	1.0a	4695de	66.8
Emamectin benzoate + Lufenuron	37.4a	3.9a	0.8a	4817e	71.1
Azadirachtin	57.1c	8.2c	2.5a	3947b	40.2
Control	75.2d	14.7d	13.7b	2815a	
Mean	50.8	7.8	3.6	4168	
Cv%	23.8	27.1	42	11.82	
F-value	31.48	18.9	9.7	49.4	
Prob	<0.001	<0.001	<0.001	<0.001	

NB. Figures within a column followed by the same letter are not significant different at Fisher's LSD test; P<0.05; <sup>†</sup>Angular transformed data

(P>0.05). In the treatment plots, the cob incidence and severity varied from 37.4–57.1% and 3.9–8.9%, respectively. Among the insecticide-treated crops, Azadirachtin treatment exhibited significant high levels of cob damage (2.5%), cob rot incidence (57.1%), and severity (8.2%) (Table 7). In comparison to maize treated with synthetic insecticide, the incidence and severity of cob rot in Azadirachtin-treated maize were 42.6 and 24.3% greater, respectively. In terms of grain yield, a significantly higher yield (P<0.001) was produced in the insecticide-treated maize compared to the untreated control. The mean gain yield (t/ha) ranged from 3341 to 4245kg/ha in the chemical insecticide-treated maize (Table 7). The highest was in Emamectin benzoate+Lufenuron-treated maize (4817kg/ha), followed by Chlorpyrifos+Cypermethrin (4695kg/ha), and the lowest among the insecticides was in Imidacloprid-treated maize (4289kg/ha). However, the latter was not significantly (P>0.05) different from the yield recorded in Azadirachtin-treated maize, 3947kg/ha. The untreated control recorded the lowest yield (2815kg/ha), about 50–60% less than the insecticide-treated maize.

### Strength of Association between FAW Infestation, Larval Count, Leaf Damage, Cob Damage, Cob Rot Severity and Grain Yield

The results of the correlation analysis indicated the FAW infestation had a significant positive correlation with maize leaf injury ( $r = 0.74^{**}$ ), mean larval density ( $r = 0.59^{**}$ ), and cob damage ( $r = 0.50^*$ ) (Table 8). However, grain yield had a significant negative correlation with every parameter assessed, including the degree of ear rot infection ( $r = -0.58^{**}$ ), leaf injury score ( $r = -0.57^{**}$ ), and percentage of plants infested ( $r = -0.54^{**}$ ). This shows that FAW infestation, leaf damage, and ear rot adversely impacted the maize grain yield. An indication that any mitigation that could lead to a reduction in any of these parameters would consequently lead to an increase in grain yield. The positive correlation among the parameters associated with FAW infestation indicates that any of them could be used as a proxy for assessing the response of FAW to treatment.

## DISCUSSION

The laboratory study results show that all tested insecticides: Imidacloprid, Spinosad, Flubendiamide, Acetamiprid, Chlorpyrifos+Cypermethrin, Emamectin benzoate+Lufenuron, Malathion, Acephate, and Fipronil were effective against the FAW and caused mortality of

above 50% 72-hour post-application period. These insecticides had been previously listed as being widely used by farmers (Kansiime et al., 2019; Ahissou et al., 2021). Percent larval mortality increased over time post-treatment, suggesting residual toxicity of the synthetic insecticides on FAW (Sisay et al. 2019). The biopesticide Azadirachtin was also able to exert over 50% larval mortality, indicating it could be a suitable alternative to any of the synthetic insecticides tested in this study. Sisay et al. (2019), working with *Azadirachta indica* and other botanicals, reported mortality of >95% 72h after application.

Timing, method of application of pesticides, and use of correct dosages are crucial for the effective control of FAW (Akeme et al., 2021; Assefa & Ayalew, 2019). For example, the most effective period is when the spraying starts early and coincides with the early to mid-vegetative growth stages, when the crop is most vulnerable to FAW attack (Nboyine et al., 2022). In the field trials, the insecticides were observed to be quite effective when applied three times, i.e., at 14, 24, and 35 days after crop emergence, using the manufacturer's recommended rates. The three sprays coincided with the early to mid-vegetative growth stages. This is when the maize crop is 28 to 42 days old which are periods when the crop is most vulnerable to FAW attack (Akeme et al., 2021). Spraying during these stages significantly reduces FAW infestation, defoliation, and larval abundance, which may otherwise affect crop productivity (Matova et al., 2020). Over 50% of infestation have been reported to occur at the early whorl stage (Akeme et al., 2021). Therefore, spraying during this period would be effective in mitigating the effects of the FAW. Nboyine et al. (2022), working in Northern Ghana, found that a minimum of two rounds of spray for insecticide (synthetic or biopesticide) adequately protected the crop against the FAW. On the other hand, Kumar & Mohan (2020) proposed that insecticides sprayed two times at a 14-day interval would effectively control FAW. The linear regression analysis of the frequency of application of the insecticides showed that, in spite of having different mechanisms of action on the FAW, there was a significant reduction in infestation with an increased number of sprays. Emamectin benzoate + Lufenuron, Chlorpyrifos + Cypermethrin, Acetamiprid, and Imidacloprid sprayed three times caused 40–70% less FAW infestation, 35–50% less leaf injury (43%), and 68.3% fewer *S. frugiperda* larval populations than the untreated control. Since the FAW larvae, especially the 3<sup>rd</sup> to 5<sup>th</sup> instar larvae,



**Table 8:** Correlation analysis of the FAW infestation, damage, leaf injury rating, incidence and severity of cob rot, and grain yield (kg/ha)

Parameter	% Plant infested	Leaf injury score	Larva/Plant	Cob damage	Cob rot incidence	Cob rot severity
% Plants infested	-					
Leaf Injury score	0.74**	-				
Larva/plant	0.59**	0.72***	-			
Cob damage	0.50*	0.54*	0.39	-		
Cob rot incidence	0.40	0.57*	0.59**	0.18	-	
Cob rot severity	0.41	0.50	0.41	0.18	0.60**	-
Grain yield	-0.54**	-0.57**	-0.48	-0.34	-0.48	-0.58**

\*, \*\*, \*\*\* Significant at  $P < 0.05$ ;  $P < 0.01$ ;  $P < 0.001$ , respectively.

feed deep in leaf whorl, when spraying, the 2<sup>nd</sup> and 3<sup>rd</sup> sprays must be directed into the whorl portion of the plants (Prasanna et al., 2018; Visser & Van der Berg, 2020).

The significant reduction in leaf damage and larval population in the treated crop compared to the untreated control could be attributed to the reduced FAW larval population in the treated plants. The FAW larval population density ranged between 0.13 and 0.4 per plant, compared to the untreated control's 0.62 and 2.06 per plant. Kumar et al. (2022) reported that mean densities of 0.2 to 0.8 larvae per plant during the late whorl stage can reduce maize productivity by 5 to 20%. Henceforth, with average larval densities of 0.62–2.06 per plant observed in the untreated maize, its likely to have suffered > 50% reduction in maize productivity. The FAW larva were significantly high in the untreated control (no spray) but decreased with increases in the number of spray applications in insecticide-treated plots.

The strong positive relationship observed between level of FAW infestation and leaf injury (0.74\*\*) and a significant negative relationship between levels of infestation and yield (-0.61\*\*) in this study suggested that the high FAW infestation in the untreated control affected plant growth and grain yield by reducing the amount of photosynthetic leaf area available for carbon fixation, affecting plant growth and grain yield. Chimweta et al. (2020) reported that intensive feeding by the FAW larvae during the growth of the maize plant may cause structural damage to the leaf whorl, significant loss in the photosynthetic area, impair reproduction, direct damage to grain, and lodging. In a study conducted in East Africa, Sisay et al. (2019) observed that leaf damage due to FAW in untreated maize was 80% more than in insecticide-treated crops. On the other hand, Hruska (2019) concluded that FAW defoliation as high as 70% at the 12-leaf stage causes a 15% grain yield loss. In this study, however, it was observed that insecticide-treated maize recorded 52–67% less damaged leaves than the untreated maize, and after the third spray, which coincided with the 12–14th leaf stage, the leaf damage in the untreated crop was 2x higher than in the treated maize, which would result in a yield loss of more than 30%.

While FAW primarily causes direct damage by feeding on leaves, leading to defoliation, it occasionally attacks developing maize ears, thereby creating entry points for fungal pathogens that cause ear rot and severely reduce grain quality (Ishola et al., 2022). At very high rates of infestation, FAW larvae may move up to the ear and feed on kernels, causing significant yield loss and creating entry points for ear rot fungi. There was a significant positive correlation between FAW infestation and the incidence

and severity of ear rots, 0.44\*\* and 0.41\*\*, respectively. The high levels of ear rot infection recorded in the untreated control could have resulted from the FAW larvae tunneling during silking and kernel development (Singh et al., 2024). The cob tunneling activity creates entry points for fungal pathogens, including ear-rot fungi. In addition, once the ear-rot enters and colonizes, the kernels produce secondary metabolites called mycotoxins in the infected cobs (Kaur et al., 2023; Njeru et al., 2020). Mycotoxins cause a range of adverse health effects in humans and livestock if ingested in large amounts. The FAW damage, which often coincides with warm and humid conditions, is ideal for fungal growth and mycotoxin production. Therefore, the combination of FAW damage and favorable weather may exacerbate ear rot development (Akeme et al., 2021; Mahmoud et al., 2024). The insecticide-treated maize effectively lowered the FAW infestation, and as a result, ear rot infection was low among the treated crop. This observation collaborates with the observations of Kaur et al. (2023), who reported that when a pesticide (synthetic or biopesticide) is applied, the treated maize has a low ear rot infection.

The effectiveness of Emamectin benzoate has been previously reported by several scientists (Mian et al., 2022; Sisay et al., 2019; Sharma et al., 2022), an observation attributable to its quick knockdown effect; no cross-resistance with any other substance in commercial use; and its mode of action being comparable to that of abamectin, a GABA- and glutamate-gated chloride channel agonist (Liu et al., 2022). Emamectin benzoate inhibits muscle contraction, causing a continuous flow of chlorine ions in the GABA and H-glutamate receptor sites by binding to several sites in the insect's chloride channels. The increase in chloride ion flux into neuronal cells results in the loss of cell function and disruption of nerve impulses. The pest is then paralyzed irreversibly, stops feeding, and mortality follows within a short period of time (Mokbel & Huesien, 2020).

The study's findings show that Emamectin benzoate, though applied as a pre-mix molecule with Lufenuron, an insect growth regulator (IGR) or insect development inhibitor, had a strong ovicidal effect (Lv et al., 2022). It consistently outperformed Imidacloprid, Acetamiprid, and Azadirachtin. Although Chlorpyrifos and Cypermethrin seemed to be just as effective as Emamectin benzoate +Lufenuron. This pre-mix effectively reduced FAW infestation, damage, and larval population on average by more than 70%, followed by another pre-mix combination, Chlorpyrifos and Cypermethrin, 67%. It is likely that the inclusion of Lufenuron enhanced the potency of the pre-mix molecule or cocktail against FAW. The superior

performance of these two synthetics, Emamectin benzoate+Lufenuron and Chlorpyrifos+Cypermethrin, could be attributed to their quick knockdown effect as a result of their contact and stomach poisoning properties and residual effect such that even after application, the FAW in the sprayed area continued to be controlled (Liu et al., 2022). According to Ahissou et al. (2021), upon being sprayed on the plant, Emamectin benzoate and Lufenuron penetrate the leaf tissue and form reservoirs within treated leaves, which provide residual activity against pests that feed on these leaves. Both Emamectin benzoate+Lufenuron, and Chlorpyrifos+Cypermethrin conferred significantly better protection than other synthetic insecticides and the biopesticide. It's worth noting that though biopesticides are environmentally safer and pose no risk to humans (Daraban et al., 2023), they are at times slow-acting substances (Iqbal et al., 2022), hence the lower efficacy observed in Azadirachtin-treated plots. For example, in some studies, Neem has been reported to cause 70–100% larvicidal in the lab (Tulashie et al., 2021; Kamunhukamwe et al., 2022; Keerthi et al., 2023), yet not be as effective in the field as in a controlled environment due to several factors. Among them, the farmer may need large quantities of the biopesticides as the concentration of the active constituents may be very variable and low to reproduce the desired effects on the targeted pest and highly UV labile, so it may have low residual effects in the field (Rioba & Stevenson, 2020).

Significantly high grain yields were obtained from plants treated with synthetic insecticides compared to biopesticides and untreated control. The insecticide treatments yielded between 10 and 30% more than Azadirachtin and 52 and 72% more than the untreated control. These results corroborate the observations made by Nboyine et al. (2022), who reported an increase in grain yields of at least 1.7-fold higher in maize sprayed three times compared to the untreated control. In another study, Kumar & Mohan (2020) reported between 14.6 and 64.8% more grain yield in the insecticide-treated maize than in untreated control. The yield was nevertheless influenced by the type of insecticide used.

While insecticide-treated maize yielded significantly more than untreated maize amidst a high FAW infestation and damage as observed in the untreated control, the concerns of insect resistance management are key in designing successful integrated pest management. Henceforth, to lessen the buildup of pesticide resistance, rotating different classes of synthetics in a spraying program must be combined with routine monitoring of pests, the use of reasonable treatment thresholds, and the full use of non-pesticidal methods such as biological and cultural control, field sanitation, and host plant resistance (Prasanna et al., 2018).

### Conclusion

The study has shown that some low-risk synthetic insecticides and biopesticides may be equally effective against FAW and that applying a minimum of three (3) sprays of insecticides such as Emamectin benzoate+Lufenuron, Chlorpyrifos+Cypermethrin, Acetamiprid, and

Imidacloprid ensures a healthy crop and may minimize FAW infestation and damage. This is not only cost-effective but also environmentally friendlier compared to where farmers sprayed chemicals for FAW control at 7-day and 10-day intervals, especially in green maize. Though a biopesticide such as Azadirachtin may be slow-acting and less effective under field conditions compared to Emamectin benzoate+Lufenuron, Chlorpyrifos+Cypermethrin, and Acetamiprid, it is a suitable alternative to more hazardous and less effective insecticides like Malathion and Orthene. Therefore, farmers planting green maize who tend to spray the insecticide weekly as a way of ensuring that the crop remains free of any blemish due to fall armyworm could effectively reduce the number of sprays and achieve the same goal. It is also important, however, that before making an insecticide spraying decision, farmers regularly monitor the FAW infestation in their maize fields. The study has provided valuable information about the efficacy of insecticides with relatively novel modes of action to manage FAW that could be included in a FAW-IPM system. Further research may be needed to test if FAW has started to develop resistance to any of the insecticides in use, including the ones tested in this study.

### Author's Contributions

Mweshi Mukanga: Conceptualization; data collection; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—review and editing Gilson Chipabika and Matthews Matimelo: Original draft preparation, review and editing, have read and agreed to the published version of the manuscript. Owen Machuku: Original draft preparation, review and editing, have read and agreed to the published version of the manuscript. Marian Lupupula, Lorraine Chilipa and Sylvia Misengo Tembo: Data collection, trial site management, review and editing. Kabosha Lwinya: Data curation; formal analysis; writing—review and editing

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