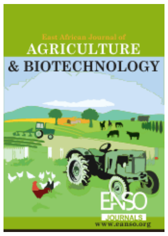

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Original Article

Integrating Entomopathogenic Fungi into Different Crop Management Practices for Fall Armyworm Management

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ABSTRACT

The study was carried out to identify crop management practices favouring entomopathogenic fungi of fall armyworm (Spodoptera frugiperda J. E. Smith). An experiment was set up with 24 maize plots measuring 6 m x 5 m in Siaya, JOOUST Campus field during the September to December 2021 short rain season for identification of crop management practices favouring entomopathogenic fungi of fall armyworm. Morphological and Molecular characterization was done to estimate the diversity of entomopathogenic fungi of fall armyworm larvae specimens from the treatment plots. Each plot was separated by 2 m occupied with Brachiaria CV Mulato to counter the possibility of fall armyworm migration from one treatment plot to another as recommended by the ICIPE's fall armyworm push-pull technology. The results showed that weeding was statistically significant (0.044) on fall armyworm larvae collected while only 2 species of entomopathogenic fungi were isolated for this crop management practice. The case was different with intercropping, though not statistically significant, over 50% of the isolated species were from this management practice. This could mean that intercropping is likely to reduce FAW larvae infestation whilst increasing fungal activity. This study therefore concludes that the threat that S. frugiperda poses also calls for the need for the development and adoption of good crop management practices such as intercropping, adequate fertilization, and weeding.

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INTRODUCTION

Fall armyworm is a dominant pest in maize production. It can cause damage by consuming the foliage of the host crop. It has now become a major invasive pest on maize in many parts of the world due to its wide host range, and strong reproductive and dispersion capacity (Li *et al.*, 2021). A study by Nwanze highlighted that FAW is one of the most destructive crop pests, with a wide spectrum of host range including maize, rice, and sorghum (Nwanze *et al*., 2021). According to the assessment by the CGIAR Research Program on maize, the FAW over the last two years caused serious damage to more than 1.5 million ha of maize crop in Africa alone negatively impacting the food security and livelihoods of many million smallholder farmers.

The problems posed by FAW can date back to over a decade ago. Without the infestation of fall armyworm larvae (FAW), yields are expected to increase up to approximately 20% improving food security (Marenco *et al.*, 1992; Ayala *et al.*, 2013). According to FAO (2020), it can cause significant damage to crops if not well managed and prefers maize, but can feed on more than 80 additional species of plants. This is especially important because maize is a staple food for hundreds of millions of people, and scientists are racing to find a solution to this pest (ICRISAT, 2018; FAO, 2020). To date, FAW infestation remains one of the major pest problems (De Groote *et al*., 2020).

In Kenya, the percentage of loss experienced by farmers as a result of FAW infestation was 54% and 42% in 2017 and 2018 respectively. In 2019 consequently, the low and medium potential maize production areas were the most affected, with reported losses of more than 50% of maize yields, resulting in a total loss of 37% for the whole country (Groote *et al*., 2020). Given this background, FAO launched the pioneering Global Action for FAW, a three-year global initiative (FAO, 2020). In addition to FAW’s emerging economic and food security impacts, there has been recent urgency to mitigate the fall armyworm problem and many methods have been introduced.

Methods of controlling the FAW exist to prevent further damage. They are categorized into chemical, cultural, botanical, and biological methods. Cultural control methods include avoiding late planting, intercropping, and rotating maize with non-host crops like sunflower and bean which may be useful to minimize the invasion of FAW (FAO, 2018). Most subsistence farmers in Africa practice cultural control methods (Abate *et al.*, 2000). A survey conducted in Ethiopia and Kenya showed that 14% and 39% of the farmers practiced cultural methods for FAW management (Kumela *et al.*, 2019). On the contrary, the botanical control method uses extracts of plants such as *Jatropha curcas*, *Nicotina tabacum*, and *Chrysanthemum cinerariifollium*.

Additionally, the chemical control method of FAW is usually achieved through the application of synthetic insecticides such as carbamates, pyrethroids, and organophosphates (Hruska & Gould, 1997; Assefa & Ayalew, 2019). This method have concerns that inappropriate use could lead to resistance development, plant damage, and risks to human health and the environment. The biological method is also used in the control of FAW and primarily focuses on restoring natural control through the action of living organisms (parasites, predators, or pathogens) introduced by human intervention.

Biological control is an effective tool and one of the most essential alternative control techniques for providing environmentally safe and sustainable plant protection (Assefa & Ayalew, 2019) and is economically viable among all the ways outlined. The larval and adult stages of FAW are easily attacked by a variety of parasitoids, predators, and pathogens. Entomopathogenic fungi are amongst the commonly used biological methods of controlling FAW. It is considered a cheap method and reported as an important agent for the control of FAW in Sub-Saharan Africa hence many farmers opt to use it (Assefa & Ayalew, 2019; Gebreziher, 2020).

The FAW outbreak has also resulted in attempts being made to integrate entomopathogenic fungi (*EPF*) into diverse cropping systems for FAW management in different areas (Otim *et al.*, 2021). The potential to control agricultural pests using EPF should not be underestimated. It dates back to as far as Augostino Bassi's 1835 who demonstrated that a fungus could cause a deliberately transmissible disease in silkworms (Steinhaus, 1956; Lord, 2007). Crop management practices contribute to either reducing pest attacks or enhancing natural enemies. The practices vary from tillage systems, and fertilizer use, to intercropping. The tillage system is one of the attempted crop management practices that has been practiced. Findings in Zimbabwe and Zambia showed that maize farmed under minimum or no -tillage significantly reduced FAW infestation on maize (Harrison *et al.*, 2019). Yet, Otim *et al*, (2021) also highlighted that minimum tillage also enhances the activities of natural enemies such as parasitoids and pathogens.

In terms of weeding manipulation, Altieri (2016) observed significantly less infestation of FAW in less weeded plots of maize compared to weeded plots, and significantly greater numbers of FAW predators were encountered. This has also been supported by a study that showed that weed manipulation enhances the population of natural enemies (Hailu *et al.*, 2018). However, despite the beneficial effect of weeds on the population of FAW pests, their infestation could cause about 20–50% yield losses in maize (Midega *et al.*, 2018). Thus, striking a balance between keeping and removing weeds is important in ensuring high farm productivity.

Intercropping practiced widely by smallholder farmers in Sub-Saharan Africa has long been recognized as an efficient farming system providing improved resource utilization and increased productivity (Abate *et al.*, 2000; Kumela *et al.*, 2019). The practice is reported to reduce FAW pest populations and enhance the potential entomopathogens (McGrath *et al.*, 2018; Babendreier *et al.*, 2020). In Latin America, maize-bean intercrops reportedly reduced FAW infestation when compared to a maize mono-crop (FAO, 2018). Similarly, studies in Uganda and Cameroon have demonstrated that intercropping maize with beans or groundnuts significantly reduces FAW infestation and damage severity in maize (Hailu *et al.*, 2018).

Further studies are required to determine the mechanism by which crop management practices reduce damage caused by the FAW and enhance natural enemy diversity (Otim *et al.*, 2021). Thus, this research incorporated different crop management conditions under a field experiment to identify crop management practices favouring native EPF of FAW.

MATERIALS AND METHODS

Study Area

The study was conducted in Siaya County, Kenya. The County comprises six (6) sub-counties: Alego Usonga, Bondo, Ugenya, Ugunja, Gem, and

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Rarieda. It is bordered by Busia County to the North, Kakamega and Vihiga Counties to the North Eastern side, and Kisumu County to the South East. The total area of the County is approximately 2,596 km² and a population of 842,325 people.

The county receives annual rainfall of between 1,170mm and 1,450mm and temperature ranges between 15 -30 degrees Celsius. The current and expected effects of climate change differ locally, nationally, and regionally affecting livelihoods, ecosystems, food security, and water availability. Consequently, the four dimensions of food security: availability, access, utilization, and stability are impacted. Current temperature and rainfall trends as well as the increasing frequency of extreme events are expected to continue in Kenya. Increased rainfall intensity will result in more frequent and severe floods, as well as lengthier dry spells mainly affecting farmers and pastoralists in the east and north of the country.

Siaya's weather patterns are increasingly changing impacting largely rainfall patterns and consequently affecting 98% of the county's agriculture which is rain-fed. The major challenge that farmers face is the timing of the onset and end of rains so as to know when to plant or harvest their crops (Ndasiyaba *et al.*, 2020). According to the Kenya Meteorological Department, the rainfall patterns are characterized by the migration of the Inter-Tropical Convergence Zone, resulting in four periods of seasonal rainfall which are: long wet season (April-June); cool dry season (July-September); short wet season (October-December) and warm dry season (January-March). The long and short rains are when most farming activities take place in almost every region of Kenya. Recent reports have highlighted that the outlook for the June-July-August 2021 rainfall season indicates that the Highlands West of the Rift Valley, Lake Victoria Basin Region, Central and Southern Rift Valley, as well as the Northwestern region, are likely to receive slightly above-average rainfall (Kenya Meteorological Department, 2021).

In Siaya, maize is grown for subsistence, alongside pearl millet, and sorghum. Dairy farming is widely practised, as well as the raising of poultry among other economic activities. Droughts have affected more people and had the greatest economic impact (8% of GDP every five years). While droughts affect most people, the effects are exacerbated by outbreaks of pests such as fall armyworms and locusts. Poverty levels have increased in recent years, especially in the densely populated central highlands, where the most intensive agriculture is practised. It contributes to people's vulnerability to climate change as it limits their social and financial options for adaptation (Kenya National Bureau of Statistics, 2020).

Experimental Design

A factorial experiment was established for this research. A 40 m by 30 m plot with subplots was set up with each plot measuring 6 m by 5 m (*Figure 1*). Each plot was separated by 2 m and Brachiaria CV Mulato was planted in the border space as recommended by the ICIPE's fall armyworm push-pull technology (Midega *et al.,* 2017). This was done to counter the possibility of the FAW larvae migrating from one plot to another. The plots were placed in a randomized complete block design (RCBD) (*Figure 1*) to account for spatial effects in the plots. Three crop management practices which are intercropping, weed manipulation, and organic manure application were used in the field experiment at 2 levels and replicated 4 times as shown in *Figure 1*. The Hybrid seed maize variety (DH 04) was used.

The FAW larvae physically infested the plots at the vegetative stage about 2 weeks after germination of the maize crop. The FAW larvae were collected at weeks 3, 4, and 5 after germination of the maize for analysis. Each sample collected was labelled as per the plot and kept in the microbiology laboratory for further analysis. The FAW samples were then morphologically and molecularly characterized to determine entomopathogenic fungal species.

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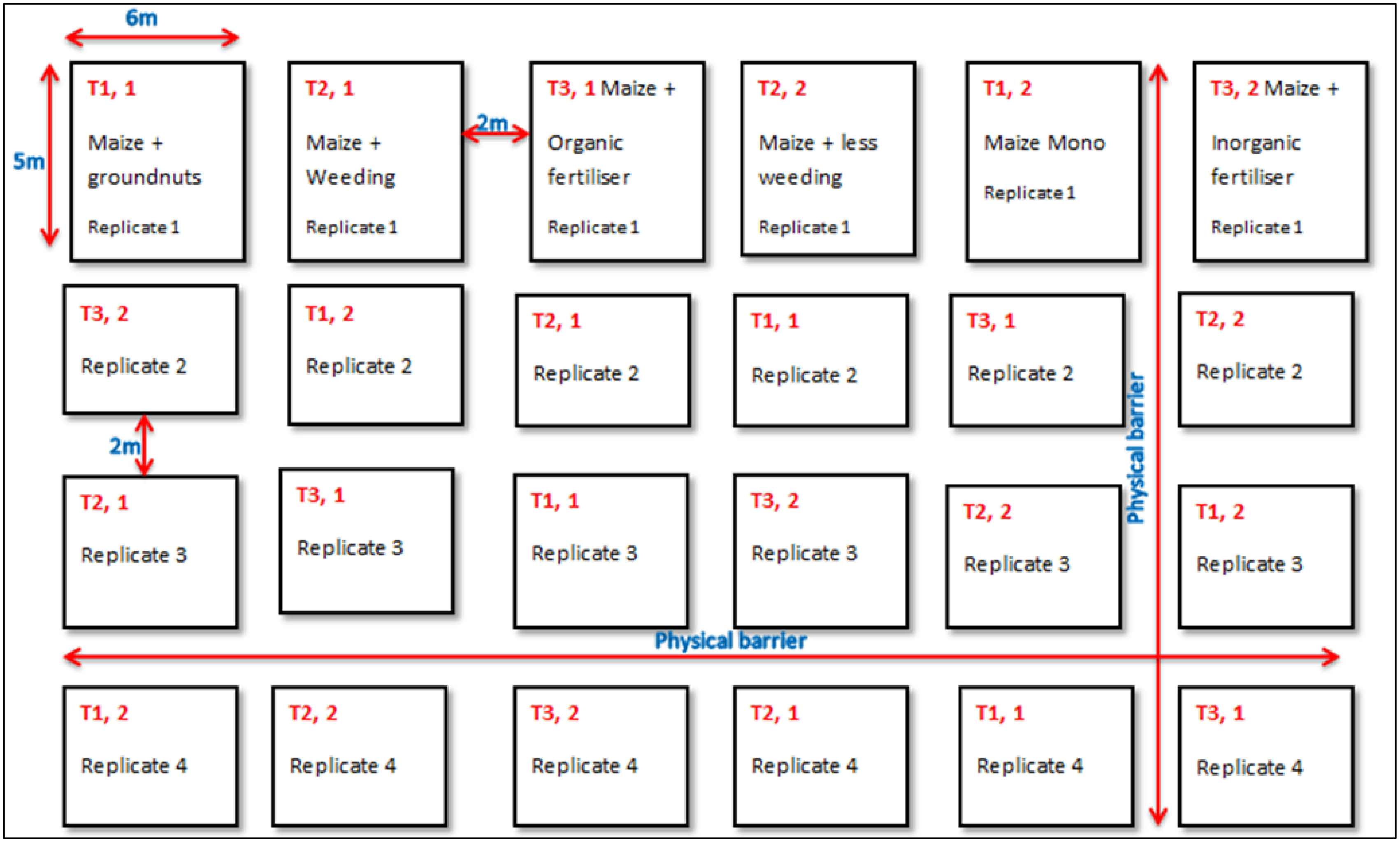
Figure 1: Field experimental design setup



Key: *T1: Intercropping vs Monocropping (McGrath et al, 2018; Babendreier et al, 2020); T2: Weed manipulation (Altieri, 2018; Kansiime et al, 2019); T3: Organic fertiliser vs inorganic fertiliser (Jabbour et al, 2009; Otim et al, 2021); Physical barrier: Brachiaria CV Mulato*

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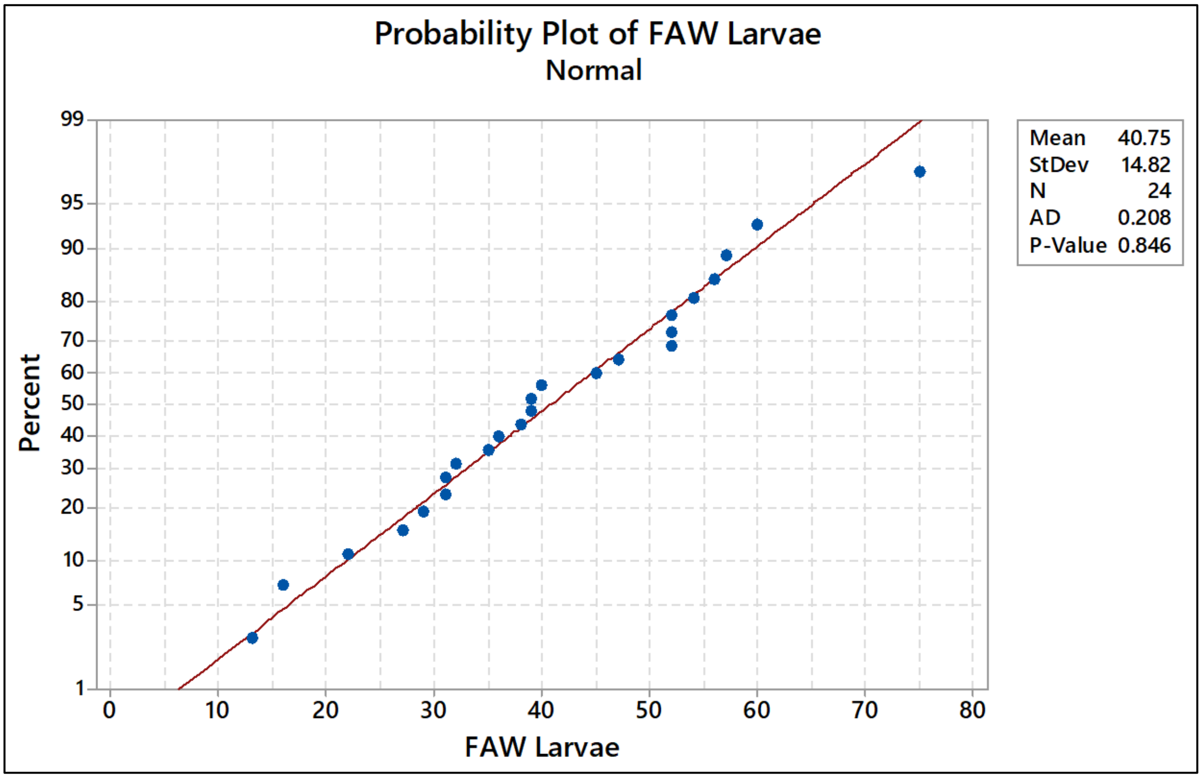
RESULTS

Normality and Pathogenicity

An Anderson-Darling test for normality showed that the data was normal (*Figure 2*). *Table 1* summarizes the crop management practices and associated fungal species observed including their pathogenicity. Blocking was also effective with a significance level of 0.201. Of all the fungal species isolated, only 1 species is known to be non-pathogenic according to literature, however, some genera such as *Irpex sp* and *Bipolaris sp* could not have 100% match identity for similarity search analysis (n = 3), as such, its pathogenicity is found in some genera.

Figure 2: Normality test



Crop Management Practices Favouring EPF of FAW

Intercropping as a crop management practice yielded 6 fungal species (32%, n = 6), followed by Monocropping (21%, n = 4). However, the least fungi species were recorded in management practices such as weeding, [1]less weeding, and organic manure (11%; n = 2) (*Table 1*). Nevertheless, the highest treatment mean for the collected fall armyworm larvae was recorded in the less-weeded plots (52), followed by inorganic fertilizer plots (48.3). Whereas, the least mean was recorded in the weeded plot treatment (28.50) (*Table 2*; *Figure 3*). Hence, there was a statistically significant difference on the less weeded plots (0.044) with a positive skewness as compared to all other treatment plots. The implication for this is that crop management practices such as less weeding may maximize fall armyworm larvae attack whilst reducing the fungi and other natural enemies' infestation.

[1] Less weeding means weeded only once in this study

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Table 1: Summary of crop management practice and fungal species

Crop management practice	Treatment	Isolated fungi species	Pathogenicity[2]	%
Intercropping vs Monocropping	Maize+ groundnuts	*Curvularia mebaldsii*	Pathogenic	32%
		Curvularia americana	Pathogenic	
		Fusarium chlamydosporum	Pathogenic	
		Talaromyces verruculosus	Pathogenic	
		Pithomyces cynodontis	Pathogenic	
		Coprinellus aureogranulatus	Non-pathogenic	
	Maize only	*Bipolaris sp*	Pathogenic	21%
		Penicillium citrinum	Pathogenic	
		Paecilomyces variotii		
		Irpex sp.	Some genera	
Weed manipulation	Maize+ weeding	*Bipolaris austrostipae*	Opportunistic pathogen	11%
		Penicillium citrinum	Pathogenic	
	Maize + non-weeding	*Pithomyces chartarum*	Pathogenic	11%
		Trichoderma harzianum	Pathogenic- natural fungicide	
Organic fertilizer vs inorganic	Maize + manure	*Trichoderma harzianum*	Pathogenic- natural fungicide	11%
		Irpex sp.	Some genera	
	Maize + fertilizer	*Aspergillus sydowii*	Pathogenic	15%
		Trichoderma lixii	Pathogenic	
		Paecilomyces variotii	Pathogenic	

Table 2: Mean summary

Treatment Plot	Mean	P-value
Fertilizer	48.3 ± 10.3^{ab}	0.529
Intercrop	39.75 ± 5.94^{ab}	0.799
Less Weed	52.00 ± 5.73^{a}	0.044
Manure	38.25 ± 7.96^{ab}	0.274
Mono-crop	37.75 ± 4.87^{ab}	0.100
Weeded	$28.50 \pm 6.61^{a\,b}$	0.875

^{ab}Means within the same row with different superscripts indicate significant differences between treatments (Fisher's LSD, $P < 0.05$).

Though not significantly different, the mono-crop treatment plots were also positively skewed (*Figure 3*). Blocking also showed variation in the experiment with block 1 having a mean of 48.33 whilst block 4 had the lowest mean of 29.50. Blocks 2 and 3 did not have much variation (*Table 2*).

[2] Pathogenicity was determined based on literature

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Figure 3: Box Plot of FAW Larvae and Treatment Plot

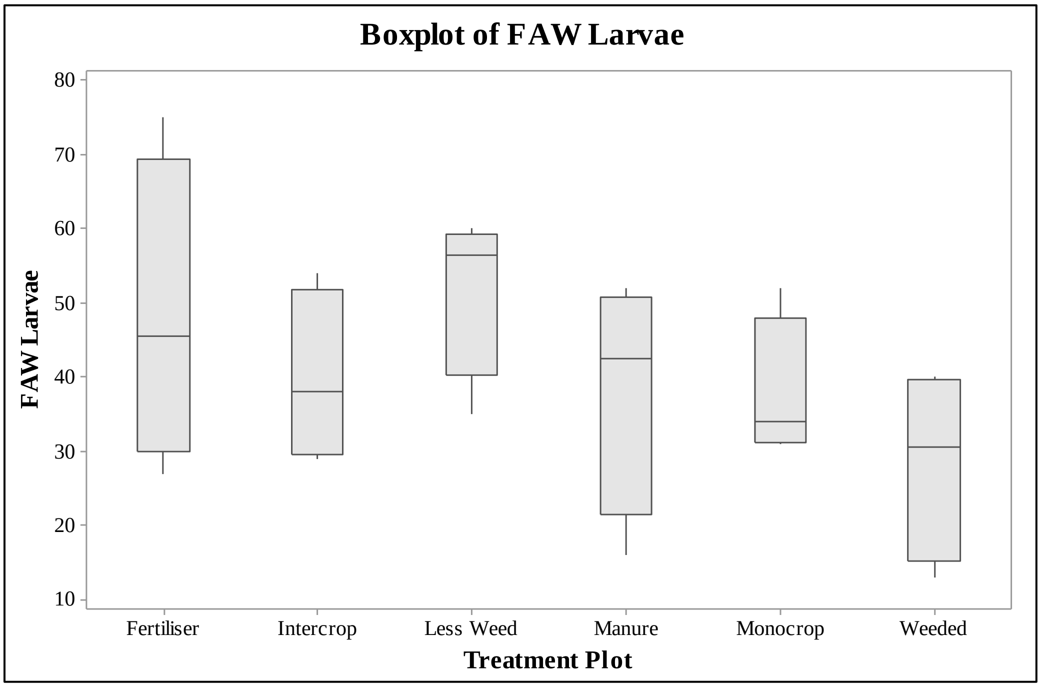

Table 3: Blocking variation

Block	Mean	SE Mean	St. Dev	Minimum	Median	Maximum
1	48.33	6.75	16.54	29.00	45.50	75.00
2	43.50	4.77	11.67	31.00	43.50	60.00
3	41.67	6.65	16.28	13.00	45.50	56.00
4	29.50	4.17	10.21	16.00	29.50	45.00

In terms of the fungi species, cross-tabulation was done to show from which treatments each species was isolated. The maize that was intercropped with groundnuts had the most isolated fungal species of all crop management practices with *Curvularia* genus (33%; n = 2) being more common. These species included; *Curvularia mebaldsii*, *Curvularia Americana, Fusarium chlamydosporum, Talaromyces verruculosus, Pithomyces cynodontis, and Coprinellus aureogranulatus* (*Table 3*). Contrary, the mono-cropped maize had 4 (four) fungal species including *Bipolaris* sp, *Penicillium citrinum*, and *Irpex sp* (*Table 3*; *Table 1*).

Additionally, the weeded maize had only 2 fungi species; *Bipolaris austrostipae* and *Penicillium citrinum* (*Table 3*) as compared to the intercropped and mono-cropped treatment plots. *Bipolaris* is known to be a natural plant pathogen whereas the latter is known to be a plant, animal, and human pathogen producing mycotoxin citrinin and cellulose digesting enzymes. Similarly, the less weeded maize treatment plots had 2 fungi species; *Pithomyces chartarum* and *Trichoderma harzianum* (*Table 1*). The latter is known to be naturally occurring and a biological control agent used against phytopathogens and bio-stimulation.

The treatment plots with organic fertilizer had 2 fungal species isolated which are *Trichoderma harzianum* and *Irpex sp* (*Table 3*). This treatment shared the *Trichoderma* with the less-weeded plots. However, the inorganic fertiliser treatment plots had 3 species of fungi isolated namely; *Aspergillus sydowii, Trichoderma lixii,* and *Pithomyces variotii* (*Table 1*). Comparatively, *Aspergillus* species were isolated uniquely to these treatment plots as the genus is known to be more virulent, and

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commercially exploited due to its ability to produce and secrete antibiotics and mycotoxins and cause many diseases in humans.

Table 4: Fungi Species vs Crop Management Practice

Fungi Species	Crop management Practice					
	Fertiliser	**Intercrop**	**Less Weed**	**Manure**	**Mono -crop**	**Weeded**
Aspergillus sydowii	1	0	0	0	0	0
Bipolaris austrostipae	0	0	1	0	0	0
Bipolaris sp	0	0	0	0	1	0
Coprinellus aureogranulatus	0	1	0	0	0	0
Curvularia americana	0	1	0	0	0	0
Curvularia mebaldsii	0	1	0	0	0	0
Fusarium chlamydosporum	0	1	0	0	1	0
Irpex sp.	0	0	0	1	1	0
Penicillium citrinum	0	0	1	0	1	0
Pithomyces chartarum	0	0	0	0	0	1
Pithomyces cynodontis	0	1	0	0	0	0
Pithomyces variotii	1	0	0	0	0	0
Talaromyces verruculosus	0	1	0	0	0	0
Trichoderma harzianum	0	0	0	1	0	1
Trichoderma lixii	1	0	0	0	0	0
Total	**3**	**6**	**2**	**2**	**4**	**2**

DISCUSSION

The pathogenicity of microbial agents can vary according to agricultural management practices and geographical location (Muhammad *et al.,* 2021). In this study, intercropping as a crop management practice yielded the highest fungal species (32%, n = 6). However, the least fungi species were recorded in management practices such as weeding and organic manure (11%; n = 2) (*Table 1*). This may imply that plant diversity in intercropping systems can reduce the rate of oviposition by confusing the FAW female moth, therefore helping reduce the level of infestation (Matova *et al.,* 2020; Tanyi *et al.,* 2020). Intercropping and other forms of plant diversity may also offer push-pull systems that help build up the population of natural enemies such as entomopathogenic of FAW (Baudron *et al.,* 2019a). In a study in Latin America, maize-bean intercrops reportedly reduced FAW infestation when compared to a maize mono-crop (Food and Agriculture Organisation., 2020). Other findings in Uganda and Cameroon have demonstrated that intercropping maize with beans or groundnuts significantly reduces FAW infestation and damage severity in maize (Hailu *et al.,* 2018). However, some studies argue that entomopathogens of FAW act from a combination of crop management practices which are; proper planting dates, fertilization, soil health, and moisture management.

Even so, the highest treatment mean for the collected fall armyworm larvae was recorded on the less-weeded plots (52), followed by inorganic fertilizer plots (48.3). There was a statistically significant difference on the less weeded plots (0.044) with a positive skewness as compared to all other treatment plots (*Table 2*). Although the less weeded treatment plots larvae collection was significant, only 2 entomopathogenic fungi (*Table 1*) were isolated. The implication for this is that poor crop management practices such as less weeding may maximize fall armyworm larvae attack whilst reducing the fungi and other natural enemies' infestation that are good for maintaining plant health. Similarly, a study carried out in Kenya by

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Mutyambai *et al.,* (2022) confirmed that maize grown under mixed cropping systems weeded frequently had a low infestation and damage from FAW compared to those grown under monoculture with no weeding. In Zimbabwe, Baudron *et al*., (2019) found out that weeding operations significantly reduced fall armyworm attacks.

However, we should be cautious with this finding because while native grasses and weeds may host natural enemies of FAW such as fungi, they may also harbour other crop pests like stem borers (Baudron *et al.,* 2019b; Harrison *et al.,* 2019) which are destructive. Hailu *et al.,* (2018) support this hypothesis with a study that showed that weeds enhance the population of natural enemies. However, despite the beneficial effect of weeds on the population of FAW pests, their infestation could cause about 20–50% yield losses in maize (Midega *et al.,* 2018). Thus, striking a balance between keeping and removing weeds is important in ensuring high farm productivity.

Though not significantly different, another important note to this study in terms of crop management practices is that fertilizer treatment plots had a fairly higher mean (48.3) and were also positively skewed (*Figure 3*). Several studies have shown the effect of fertilization on maize in FAW larval growth and mortality, but sometimes there is even a difference in the type of fertilizer (Supartha & Susila, 2022). In a study by the International Maize and Wheat Improvement Centre, the application of manure and compost was found to decrease fall armyworm damage and hypothesized to increase the abundance of natural enemies including fungi (Johnson, 2019). This is contrary to a study in Brazil that established that chemical fertilizer resulted in significantly higher levels of FAW infestation in maize than treatments with no fertilizer used, or organic fertilizer.

CONCLUSION AND RECOMMENDATION

The threat that FAW poses calls for the development and adoption of good crop management practices such as intercropping, adequate fertilization, and weeding. These practices stood out in this study, in terms of either reducing fall armyworm larvae infestation or enhancing more fungal activity. Amongst the identified entomopathogenic fungi, *Aspergillus*, *Curvularia* and *Penicillium* species identified in this study are pathogenic against *S. frugiperda* as is supported by a lot of studies. These species can therefore be used when employing bio-control methods against fall armyworm larvae for improved maize productivity and ensuring a step towards food security.

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