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Effectiveness of native entomopathogenic fungi against fall armyworm (*Spodoptera frugiperda* J.E Smith) in Kenya

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Abstract

The fall armyworm (FAW) *Spodoptera frugiperda* (J.E Smith) is an important pest of some of the major crops grown around the world. Its control is mainly by use of insecticides which with frequent use pose risks to human health and the environment. The objective of this study was to determine the potential of using entomopathogenic fungi (EPF) as a biological control for this pest. A laboratory experiment was carried out at Kenya Agricultural and Livestock Research Organization (KALRO)-National Sericulture Research Centre (NSRC), Thika where field-collected entomopathogenic fungi (EPF) were tested for their efficacy against FAW. A fungal suspension was made from fall armyworm cadavers collected from different parts of the country in 2018 and used to infect healthy FAW. The results showed that native fungal isolates could cause mortality of the fall armyworm caterpillars. The mortality of the fall armyworm caterpillar started five days after treatment. The cadavers were covered with white mycelia resulting in a characteristic white muscardine appearance and causing 69% mortality. The results reveal presence of native entomopathogenic fungus in the country, which can be exploited for development of an effective bio-control agent for the management of the fall armyworm.

Keywords: *Spodoptera frugiperda,* entomopathogenic, bio-control, silkworm, mortality, mycelium

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Introduction

The fall armyworm (FAW) *Spodoptera frugiperda* (J.E Smith) is an important insect pest causing economic losses to crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), groundnuts (*Arachis hypogea* L.), cotton (*Gossypium hirsutum* L.), soybeans (*Glycine max* L.) and many others (Sparks, 1986). The pest is new to Africa and it was first confirmed in West Africa in early 2016 (Goergen *et al*., 2016). In Kenya, the pest was first detected in maize farms in Trans-Nzoia County in March, 2017 and it has continued to spread in all the maize growing regions across the country (MOA, 2017).

In the absence of proper control methods, FAW has the potential of causing maize yield losses of 8.3 to 20.6 million metric tons per year, in 12 African countries producing maize (Abrahams *et al*., 2017). This represents a range of 21-53% of the annual production of maize averaged over a three-year period in these countries. The value of these losses was estimated at between US$2.48 billion and US$6.19 billion (Abrahams *et al*., 2017). Maize production in Kenya was expected to decline by 24.3 percent to 28 million bags in 2017 from 37 million bags in 2016, against the country's food security requirement of 40 million bags due to the invasion of FAW (MOA, 2017).

Currently FAW management is mainly by use of chemical insecticide and the persistent use of these products has led to the development of resistance in the pest population, reduction of the natural enemy population, pest resurgence and environmental contamination (Yu *et al*., 2003). With the current global concern on health and environmental pollution, there is need for evaluation of an alternative control method of this pest. Several organisms have shown the potential of reducing populations of the FAW. These are natural enemies represented by parasitoids, predators and pathogens (Murúa *et al*., 2009). Among the bio-control agents currently in use, entomopathogenic fungi have been reported to possess unique ability to attack all the development stages of insects including eggs (Hajek and St.

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Leger, 1994). However, in Kenya, no studies have been carried out to assess the effectiveness of EPF. Therefore, the aim of this study was to determine the potential of locally available entomopathogenic fungi for the control of the FAW.

Materials and methods

The FAW larvae infected with an unidentified entomopathogenic larvae were collected from a maize field in Bomet County in March, 2018. The larvae were picked from whorl-stage maize and brought to KALRO-NSRC laboratory in Thika. The NSRC laboratory is situated within 1°03'S and 37°08'E with an average annual rainfall of about 850mm and average annual temperature of 19°C. The area lies at an altitude of 1477m above sea level (Jaetzold *et al.*, 2006).

The cadavers of the FAW from Bomet County which displayed signs of fungal infection was grounded and mixed with 10ml of water. Six healthy larvae were dipped into the suspension and put in a plastic container measuring 14cm×14cm×6cm lined with wet filter paper and covered with a muslin net. The larvae were fed on fresh maize leaves and stalk daily while monitoring the mortality due to the applied suspension of entomopathogenic fungus. After 14 days, two of the healthy larvae died displaying the same symptoms as the originally infected larvae from Bomet County.

Test of pathogenicity

The two cadavers were ground using a pestle and mortar to make a suspension that was used to confirm the cause of death. 10ml distilled water was added and 26 healthy larvae of different instars were dipped inside the suspension for about 30 seconds each and placed individually in 300ml plastic container for observation. They were fed with fresh maize leaves and stalks on a daily basis. The larvae used were from a pure colony reared at the KALRO-NSRC laboratory. Data collection was done for a period of 14 days.

The same trial was done on silkworm larvae *Bombyx mori* and data was collected for 21 days for comparison. Silkworm is a lepidopteran that is

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susceptible to various entomopathogens and is used as a general control due to its sensitivity to infections. This silkworm is fully domesticated insect for purpose of silk production.

Rearing of fall armyworm in the laboratory

Rearing of the fall armyworm used in this experiment was done at room temperature. The collected larvae were kept individually in 300ml translucent plastic container lined with sterile filter paper, covered with muslin net over and held on with rubber band to prevent the larvae from escaping. The larvae were fed on fresh maize stalk and leaves on a daily basis. When they pupated, sexing was done and the pupae were kept separately until they emerged as adults. After emergence, pairing was done and each pair consisted of a male and a female. The adults were fed on honey diluted with water at a ratio of 30:70 to ensure their survival until egg laying. When the eggs were laid, they were separated from the adults and kept in a separate container to allow them to hatch. This was under room temperature (25^{o}C and prevailing room relative humidity of 65%). After hatching, the neonate larvae were kept in a rectangular plastic box measuring 25cm×18.5cm×10cm and fed on young tender maize leaves and stalks until they reached the 4th instar. At this stage the larvae were separated and kept individually because of their cannibalistic nature until they pupated. The pupae were reared until adults emerged, pairing was done in order to get a new generation.

Results

Efficacy of entomopathogenic fungus on *Spodoptera frugiperda* larvae

A total of 18 larvae died showing signs of fungal infection while five died without exhibiting any signs of fungal infection. Three of the live larvae pupated showing that their developmental processes were not affected. The cadavers displaying symptoms of fungal infection were covered with a white fungal growth. Mortality that could be attributed to fungal infection was computed at 79.6% while 8.9% was due to unknown causes. The remaining 11.5% were healthy (Table1).

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Table 1: Efficacy of entomopathogenic fungus on *Spodoptera frugiperda* larvae

Number of larvae	Instar inoculated	Number of dead larvae days after treatment										
		4	5	6	7	8	9	10	11	12	13	14
4	1st	1	1	0	2	-	-	-	-	-	-	-
2	2nd	0	0	1	1	-	-	-	-	-	-	-
17	3rd	0	0	1	1	4	5	2	2	-	-	-
2	4th	0	0	0	0	1	0	0	1	-	-	-
1	6th	0	0	0	0	0	0	0	0	0	0	0
26	total	1	1	2	4	5	5	2	3	0	0	0

Plate 1: Picture of FAW larva showing signs of fungal infection

*Date inoculated with EPF= 09/04/2018

Efficacy of entomopathogenic fungi (EPF) against different instars of the fall armyworm

Different instars of the FAW were used for the trial. Second and fourth instars suffered the highest mortality due to fungal infection of up to 100%. The 1st instar recorded a mortality of 75% due to fungal infection and 25% due to unknown cause. The 3rd instar had 65% mortality due to fungal infection, 24%

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due to unknown causes and 11% free from infection. The only 6th instar larva used in the study survived up to adult stage (Figure 1).

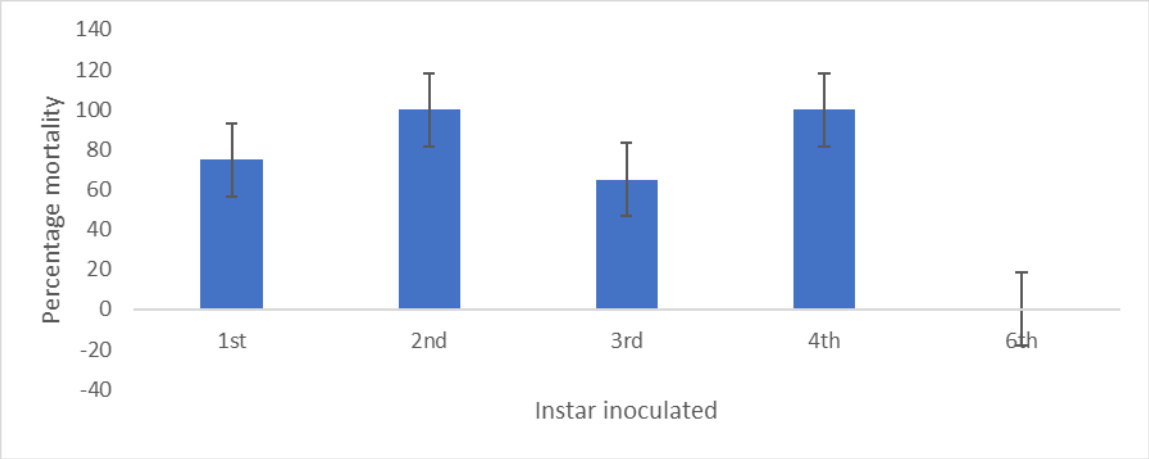

Figure 1: Efficacy of EPF against different instars of the fall armyworm

Efficacy of EPF on silkworm (*Bombyx mori*) larvae

Out of the 26 silkworms, 19 of them died and their cadavers were covered by white mycelium, one died due to unknown cause and six of them pupated and later developed up to adult stage. This represents 73.08% deaths due to fungal infection, 3.84% death due to unknown cause and 23.08% free from infection.

Plate 2: Picture showing infected Bombyx mori caterpillars infected by EPF

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Table 2: Efficacy of entomopathogenic fungus on silkworm (*Bombyx mori*) larvae

Number of larvae	**Instar inoculated**	**Number of dead larvae days after treatment**								
		9	10	11	12	13	14	15	16	17
26	4th	0	1	7	8	1	1	0	0	2

Date inoculated with EPF=21/04/2018

Further analyses using various rates of the isolate from Bomet show that the EPF at the rate of 3ml/10ml water (2nd instar larva) followed by 5, 2, and 4 (2nd instar larvae) per 10 ml of water were the most pathogenic rates with each recording a Bombyx larval mortality of 90, 85, 80 and 65 % as compared with rate of 1 (2nd instar larva per 10 ml of water and the untreated control respectively (Table 3).

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Table 3: Efficacy of different Entomopathogenic Fungi (EPF) isolates from Bomet against 20 Bombyx larvae inoculated on 31/7/2018.

Code No.	Rate of Application/ 10 ml of Water	Number of dead larvae														Total Dead after 10 days	Total Dead after 14 days	% Mortality At 10 days	% Mortality At 14 days
		Days after inoculation (infection)																	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**				
A	3 (2nd Instar Larva)	0	0	1	0	0	4	0	4	7	2	0	0	0	0	18	18	90	90
B	5 (2nd Instar Larva)	0	0	0	0	0	2	0	9	6	0	0	0	0	0	17	17	85	85
C	4 (2nd Instar Larva)	0	0	0	0	0	1	1	0	8	3	0	0	0	0	13	13	65	65
D	2 (2nd Instar Larva)	0	0	1	0	0	0	1	2	4	8	0	0	1	0	16	17	80	85
E	0(2nd Instar Larva) Control	0	0	0	0	0	0	0	0	0	2	0	0	8	4	2	14	10	70
F	1 (2nd Instar Larva)	0	0	0	0	0	0	0	1	0	2	0	0	13	1	3	17	15	85

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The EPF isolate from KALRO Katumani was exposed to 226 worms (Figure 2). Out of these worms, 176 died as a result of the infections. Only 7 worms pupated but none emerged as adult, implying that the EPF caused decimation of these worms.

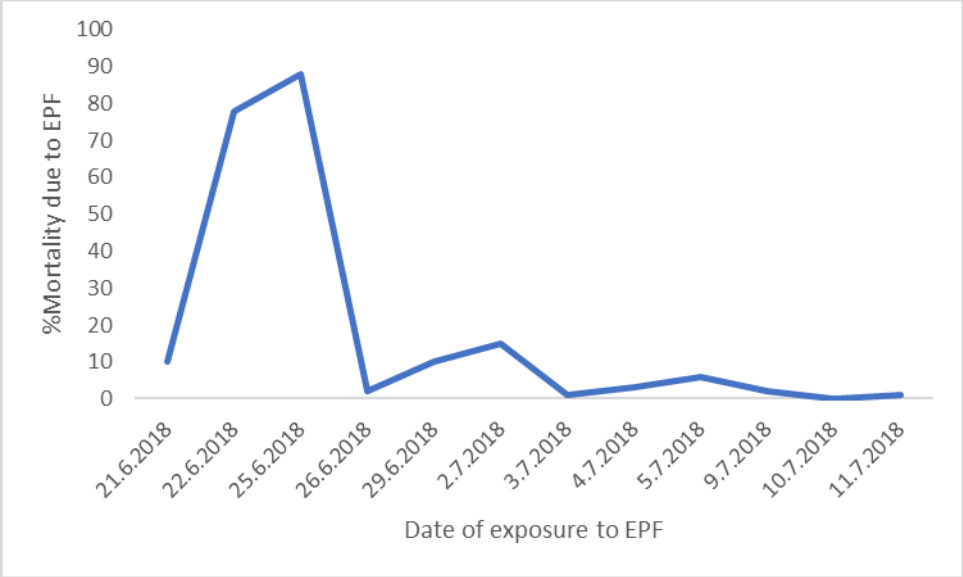

Figure 2: FAW larval mortality exposed to EPF isolate collected from KALRO Katumani.

Discussion

The results obtained from this study corroborate findings from other parts of the world such as an experiment carried out in Mexico where EPF was responsible for about 69.54% death of the fall armyworm larvae in the laboratory (Murúa *et al.*, 2002) and 4.42% larval mortality was due to unknown causes (Ruiz-Nájera *et al.*, 2013).

The early instars died within a short time compared to the older larvae, which may be attributed to the fact that their cuticles are still soft making it easier for the fungus to penetrate as compared to the mature instars whose cuticles were hardened. The entomopathogenic fungi are known to cause mortality of the insect through various ways. For example, once they attach themselves to the insect cuticle, their conidia will start to grow,

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producing enzymes that break down the insect's chitin-protein matrix. The fungi will then use a mechanical process to break through the cuticle and invade the insect's body. It also secretes toxic secondary metabolites that help it invade the insect's hemolymph, proliferates throughout the insect's body cavity, resulting to the mortality.

The first silkworm mortality occurred on the 10th day and mortality of up to 73% due to fungal infections were recorded. These results are similar to a bioassay study carried out in Nepal where the highest mortality of silkworm larvae infected with fungi was observed on the 10th day and a mortality of 70% recorded (Pokhrel *et al.*, 2014). The higher rate of silkworm death may be attributed to the fact that the worms are normally susceptible to infections and could not survive without being domesticated under highly hygienic conditions.

Conclusion

The study found out that EPF has a high potential as a bio-control agent against the FAW in Kenya. The fungi can be used in the manufacture of bio-fungicide which can be used as an insecticide for the control of *S. frugiperda* and other insect pests which are susceptible to the fungi. The early instars were more susceptible than the older instars. This knowledge can be used to inform the stage of instars that should be targeted while administering the bio-fungicide. The bio-fungicide needs to be applied on early instars of FAW so as to reduce the population of the pest effectively. Further, it is possible to apply these bioagents at earliest when signs of infestation are noted. This will ensure that young worms get in contact with the fungal spores.

Recommendations

Further studies should be carried out to identify the species of fungi that caused significant mortality of the FAW in the field. More laboratory bioassays should be carried out to validate the experimental fungus and also to determine the correct dosage of the fungi that can cause highest mortality of the larvae within the shortest time possible. The fungi can be used to make

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a bio-fungicide which can be used in the management of the FAW.

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