Journal of Life Science and Biomedicine
Volume 13, Issue 1: 17-24; March 25, 2023
DOI: https://dx.doi.org/10.54203/jlsb.2023.3

Evaluating the effectiveness of isolated fungi against the Fall Armyworm (*Spodoptera frugiperda*)

Abera Hailu DEGAGA[1, 2], and Emana Getu DEGAGA[1]

[1]*Department of Zoological Sciences, College of Natural and Computational Sciences, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia*
[2]*Department of Wildlife and Ecotourism Management, College of Agriculture and Natural Resource, Wolkite University, P.O.Box 07, Wolkite, Ethiopia*
Corresponding author's Email: aberaabos@gmail.com

RESEARCH ARTICLE
PII: S2251993923000003-13
Received: January 24, 2023
Revised: March 24, 2023
Accepted: March 25, 2023

ABSTRACT: ***Spodoptera frugiperda*, also known as the fall armyworm (FAW), is a polyphagous, destructive, and internationally dispersed insect pest that poses a serious danger to the production of cereals in Africa. Entomopathogens are a safe and environmentally beneficial way to control insect infestations when the all types of insecticides being used to control FAW are linked to a range of serious human health problems from respiratory to cancer. The objective of this study was to identify fungi from local soil that were effective against *S. frugiperda* larvae. Aluminum foil was used to gather soil samples from various maize growing regions in Ethiopia. Fungi isolated from soil samples and suspended conidia preparation was done in the mycology lab of Addis Ababa University in Ethiopia. At Melkasa Agricultural Research Center a bioassay test was conducted on *S. frugiperda* larvae. Three replications and a fully randomized design was used to set up the six treatments for the experiment. Twenty FAW larvae (3rd instar) were put into sterile Petri dishes with a diameter of 9 cm and a filter paper lining. A new, unopened syringe was used to inject 3 ml of the suspended solution into each treatment. As a negative control, distilled and sterilized water was used. FAW larval mortality was measured and examined independently for each treatment using GLM, followed by a Tukey's HSD post-hoc test. The cumulative mortality rate (P = 0.0001) and second-day mortality rate (P = 0.001) showed a significant difference between regimens. These noteworthy variations were seen in third instar larvae. *S. frugiperda* larvae from isolation fungi F1 and F5 had the highest (96.67%) and lowest (80.0%) mean cumulative death rates. Under negative control, the mean cumulative mortality rate was 8.33%. The outcomes showed that *S. frugiperda* could potentially be controlled by fungal isolates found in maize fields. Conidial concentration, as well as field evaluation, characterisation, and species identification of isolate fungi, all require more study.**

KEYWORDS: Entomopathogens, Fall armyworm, Isolate fungi, Polyphagous, Environmental health

INTRODUCTION

Most insects are helpful to humans, but a tiny number are pests or disease carriers that have an adverse impact on food supply, human health, and welfare. Due to its capacity for natural dispersal and global trade, Fall Armyworm (FAW) (*S. frugiperda*, Smith) is a destructive and transboundary insect pest with a high potential to spread [1]. The Americas' tropical and subtropical climates are home to this lepidopteran insect problem [2,3]. FAW saw the first time outside of its natural habitat in West Africa in 2016 [3-6]. After that, it was discovered in the majority of Sub Saharan African nations [3, 7], and it quickly moved to Eastern Africa [8]. FAW quickly and naturally spread upon its arrival and now routinely infests millions of hectares of maize farms across Africa and Asia [9]. In large parts of Africa, Asia, and some parts of Europe, environmental conditions support the permanent establishment of FAW [10, 11]. Tefera et al. [12] suggested that due to the presence of abundant suitable host plants and ideal climatic conditions in the regions, the pest can produce several generations in a season.

FAW undergoes complete metamorphosis and passes four developmental stages (eggs, six larval instars, pupa, and moth) [10]. It has a wide range of host plant species in many tropical and temperate regions. FAW larvae can feed on more than 350 plant species, including maize, rice, sorghum, millet, sugarcane, vegetable crops, and cotton [13]. *S. frugiperdais an* important pest of cultivated cereals like maize (*Zea mays*), sorghum (*Sorghum bicolor),* and pearl millet (*Pennisetum glaucum*) [3, 14]. *S. frugiperda* has become a threat to grain production on the African

continent [15]. Different African countries reported different amounts of maize yield loss due to FAW infestation: in Zimbabwe, from 32 to 48% [16]; in Ghana and Zambia, from 22 to 67% [17]; in Ethiopia, 32%; and in Kenya, 47% [18]. Currently, in Ethiopia *S. frugiperda is* considered as an invasive insect pest in maize farming.

FAW impacts can be minimized by implementing different control methods such as pesticides, biological, cultural/mechanical, and integrated pest management techniques [3]. Chemical insecticides are heavily used in various countries around the world to control FAW [18]. Applying chemical insecticides affects the environment, biodiversity, and health of producers negatively [19], and frequent application leads to the development of insecticide resistance and secondary pest outbreaks [15, 20]. Therefore, developing ecofriendly FAW control methods like biological ones is necessary. One of the biological control methods is to use entomopathogens. Entomopathogens include non-cellular agents (viruses), prokaryotes (bacteria), eukaryotes (fungi and protists), and multicellular animals (nematodes). FAW larvae are susceptible to entomopathogenic microorganisms such as fungi, bacteria, viruses, and protozoa [21-23], which infect and cause disease in insects [24]. Those entomopathogens reduce crop damage directly by killing insect pests and reducing their feeding habits. This study focused on fungi because they are widely distributed in the soil environment and have different ecological functions. Entomopathogenic fungi were used as a key component of integrated pest management (IPM) strategies for FAW control [25]. Insect mycopathogens enter through the cuticle [26].

Different isolate fungi's virulence and pathogenicity to insect pests differ genetically across biogeographic strains [27]. Fungi could be isolated from different stages of the pest eggs, larvae, pupae, or adults. But the known entomopathogen fungi species of the genera Metarhizium and Beauveria are commonly found in the soil [28]. A study in the laboratory by Shahzad et al. [29] found that *B. bassiana*and *M. anisopliae* were effective controls against early instar FAW. In Ethiopia, isolates of both *Metarrhizium* and *Beauveria* spp. were tested against FAW [8]. Further study on isolating fungi from the host niche helps develop a safe, eco-friendly, and sustainable bio-insecticide for managing FAW. Therefore, the objectives of the study were to isolate locally available fungi from maize-growing area soil and test their effectiveness against *S. frugiperda* larvae under laboratory conditions.

MATERIALS AND METHODS

Twenty soil samples were taken from various maize and sorghum growing regions in Ethiopia, and their GPS coordinates were recorded. Soil samples were stored in aluminum foil to prevent any potential contamination. Then, for ease of use during culturing and data collection, each sample was coded. At Addis Ababa University's (AAU) Mycology Laboratory in Addis Abeba, Ethiopia, fungi were isolated and conidia were suspended. The *S. frugiperda* colony was acquired from the Melkasa Agricultural Research Center's (MARC) plant protection laboratory in Melkasa, Ethiopia. At the MARC insect rearing laboratory, parasitoid and disease free FAW larvae were raised using its natural host, young maize stems and leaves. Third instar FAW larvae were used to test the effectiveness of isolated fungi.

Fungi isolate preparation

For culturing on prepared media, collected soil samples were diluted using distilled and sterilized water. Fungi were grown through serial dilution procedures. The plate was made using Potato Dextrose-Agar (PDA) media according to the user's manual (Blulux Lab. Ltd., India). The measured PDA was diluted with distilled water, then boiled and autoclaved at 121 $^{\circ}$C for 21 minutes. The prepared media was dispensed on a clean, autoclaved petri dish (9 cm in diameter) inside the laminal hood and left off for solidification. In the prepared petri dish, the isolated fungi were inoculated and kept upside down. The cultured petri dishes were incubated randomly for a week; dead and contaminated cultures were discarded. This procedure was repeated until the culture was completely pure. Five pure cultures were selected for further purification. After a week, those purified cultures were kept in a refrigerator at 4 $^{\circ}$C for further culturing and to harvest conidia. Before the first two weeks of the bioassay test, pure culture suspensions were made.

Figure 1. Fungal conidia harvesting under sterile conditions inside Laminal Hood in the Mycology Laboratory of Addis Ababa University (Photo by: Abera H.)

Figure 1 shows the sterile procedure for collecting fungal conidia inside the laminal hood. After flooding the plates with distilled, sterile water, 14 days old sporulation cultures' surfaces were gently scraped. Conidial

suspension was transferred to a sterile 50 ml centrifuge tube after being filtered through several layers of sterile cheesecloth. Before inoculation, the suspension was homogenized for two minutes with a magnetic stirrer.

The bioassay test experiment was arranged in a complete randomized design (CRD) with six treatments (five isolated fungi (F1, F2, F3, F4, and F5) and one negative control (C)) with three replications. Twenty FAW larvae (3rd instar) were transferred into clean petri dishes (9 cm in diameter) lined with filter paper for each replication. Three milliliters (ml) of a suspended solution of isolated fungi were inoculated on the prepared petri dish with a newly opened syringe. As a negative control, 3 ml of distilled, sterile water was applied. Clean and fresh maize leaves and young stems were provided as feed. Feed was changed every third day after waste and leaf debris had been cleaned. Petri-dishes containing treatments with FAW larvae were labeled and sealed with masking tape, then arranged in a CRD for incubation. The insectary room temperature and relative humidity were kept as needed; RH was kept by using water-soaked cotton wool. Mortality of FAW larvae was recorded daily until pupation started. The mortality rate of FAW larvae for each treatment was computed separately using the formula:

$$\%\ Mortality = \frac{No\ of\ Dead\ FAW\ Larvae}{Total\ No\ of\ FAW\ Larvae} * 100$$

Data analysis

Using MS Excel, a graph was created showing the mean mortality rate for each day and the cumulative mortality rate for each treatment. Following individual GLM analyses of each day and cumulative mortality rate of FAW larvae per treatment, post hoc tests based on their mean difference were performed. Using the Tukey's HSD test with a 95.0% confidence interval, pairwise comparisons were made for the cumulative mortality rate of FAW larvae on the second day and between treatments (CI). The statistical program SPSS 24.0 was used for the analyses.

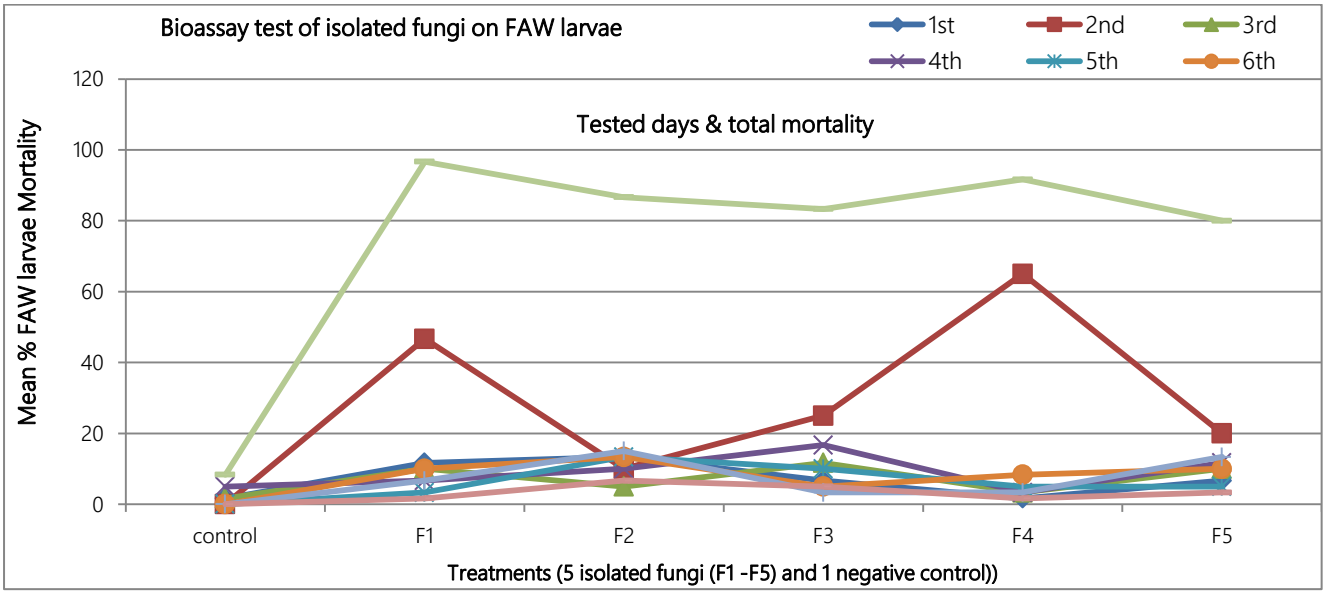
RESULTS AND DISCUSSION

Out of the twenty soil samples collected, we obtained five pure isolates of fungi. The mean mortality rate of bioassay-tested FAW larvae per treatment for each day is presented in Graph 1. In all treatments, the highest mortality rate was recorded on the second day after the isolates were inoculated. The second-day mean mortality rate of FAW larvae was greater than 20% for all isolated fungi except isolate F2 (10%). On this day, the highest mean mortality rate recorded was 65.0% and 46.67% for isolates F4 and F1, respectively, with no mortality for the negative control. On other days, the mean mortality rates for all treatments were less than 20%. The highest mean cumulative mortality rates of 96.67% and 91.67% were recorded for isolates F1 and F4, respectively. This shows that isolates F1 and F4 have higher efficacy than the other isolates. A similar result was reported by Rajula et al. [30], who isolated fungal cells after twelve days of inoculation that caused a mortality of 91.67% on *S. frugiperda* larvae. In laboratory studies, *Metarrhizium anisopliae* isolate caused 97% mortality in FAW neonate larvae [31].

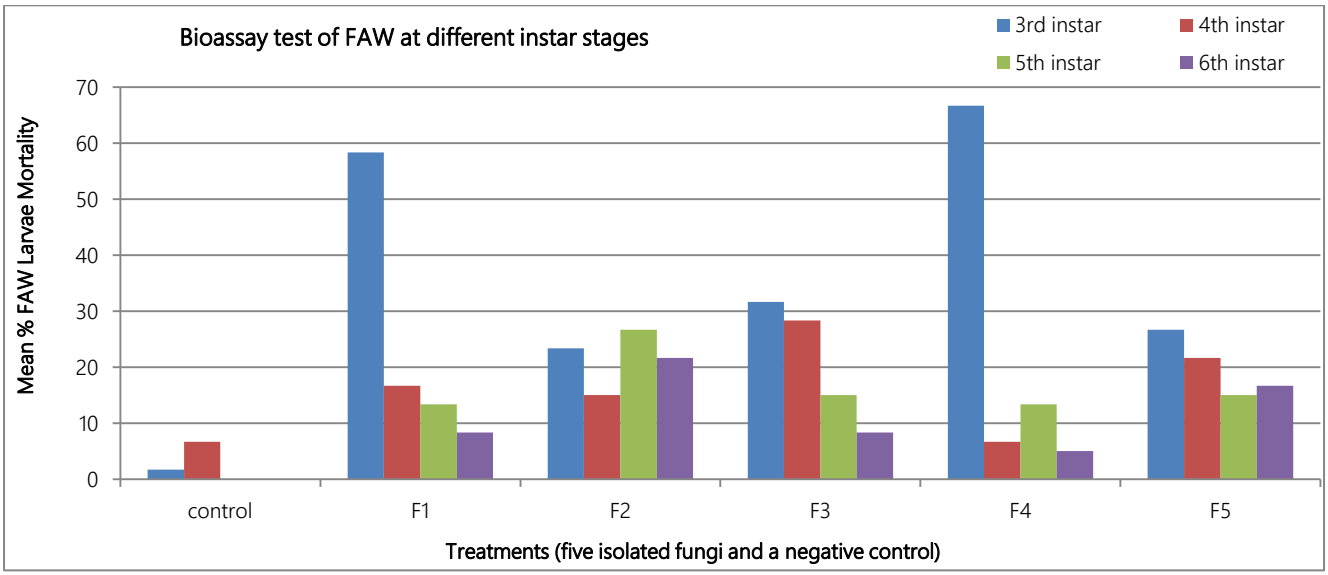
Graph 2 describes the bioassay test results at different FAW larvae instar stages after inoculating the isolate fungi. Mortality of FAW larvae at third and fourth instars was recorded in all treatments, including the negative control. Third-instar FAW larvae inoculated with isolate F4 fungi for the first two days had the highest mean mortality rate of 66.7%. The lowest was 23.3% for F2 fungi on the same instar stage (3rd). This finding is supported by Ramanujam et al. [32] and Shahzad et al. [29], who discovered that entomopathogenic fungi have a high mortality rate at an early stage. Similarly, Rajula et al. [30] found that after the third day of application, the isolated fungi killed approximately 43% of *S. frugiperda* larvae. High mortality rates were also reported by Ramirez-Rodriguez and Sánchez-Pea [33] for *B. bassiana* isolates from soil, which caused 98.3% mortality in third-instar *S. frugiperda* larvae. Idrees et al. [34] also reported that fungal isolates were less potent in reducing the feeding activity of fourth- to sixth-instar *S. frugiperda* larvae. During our study, FAW larvae of the 1st to 6th instars stayed for 3, 3, 2, 2, 2, and 2.5 days, respectively, for a total of 14.5 days. This finding is similar to that of Pitre and Hogg [35], who found that FAW larvae reared at 25 °C had mean development times of 3.3, 1.7, 1.5, 2.0, and 3.7 days for the first to sixth instars, respectively.

There were significant differences among treatments in the mean cumulative mortality rate of tested FAW larvae before pupation ($F = 57.11$; $DF = 5$; $P = 0.0001$) and the second-day mortality rate ($F = 10.35$; $DF = 5$; $P = 0.001$). Those significant differences were observed on the second day in the third instar larva of *S. frugiperda* but not in the rest of the instars (Table 1). The results of the post-hoc test for isolated fungi for the second day of mortality rate revealed a significant difference ($\alpha = 0.05$) between the isolate fungi F1 and F2; F2 and F4. However, there were no significant differences between isolates of F5 fungi with all treatments (F2 and F3) or among F1, F3, and F4 fungi (Table 2). During this time, FAW larvae mortality rates ranged from 65.0% for isolates of F4 fungi to 10.0% for isolates of F2 fungi.

Table 3 displays the findings of post-hoc analyses of the cumulative mortality rate of FAW larvae per treatment. A significant difference between the letters in a column is at α= 0.05. There was a significant difference between them; the cumulative mortality rate of FAW larvae reached as high as 96.67% for isolate F1 fungi and the lowest was 80.0% for isolate F5 fungi. Similar results were reported for Mexico by Cruz-Avalos et al. [36], who found that entomopathogenic fungi caused 97–100% FAW larval mortality. However, a Chinese study found no discernible differences between the isolated fungi and first to sixth-instar *S. frugiperda* larvae in terms of susceptibility to infection [34]. These variations may result from genetic variations in fungi or FAW larvae. Agricultural practices and location can also affect the pathogenicity of microbes [29]. However, the isolate fungi F1, F2, F3, and F4 and F2, F3, F4, and F5 do not significantly differ from one another. The mean cumulative mortality rate of the negative control was significantly lower than that of all isolate fungi. According to FAO [3] the majority of biological pesticides reduce pests' appetites rather than immediately killing them. This lessens crop damage and quickly kills insect larvae after exposure. According to Hassan et al. [37] mortality rates of 60% and higher are sufficient for controlling insect populations, as opposed to the WHO's [38] definition of treatment effectiveness, which required a mortality rate of at least 85%. It was demonstrated that fungi isolated from the soil could be bio-insecticides against *S. frugiperda* based on this mean cumulative mortality rate of FAW larvae. In light of their cumulative mortality rate, F1, F2, and F4 are therefore regarded as effective entomopathogenic fungi (Table 3). The control of insect pest populations is aided by the presence of entomopathogenic fungi in the field.



Graph 1. Mean Total Mortality Rate of FAW larvae exposed to different isolate fungi (F1, F2, F3, F4 and F5) and negative control per tested days after inoculation.



Graph 2. Mean mortality rate of FAW larvae from 3rd to 6th instars vs. treatments (five isolated fungi and negative control).

Citation: Abera HD, and Emana GD. Evaluating the effectiveness of isolated fungi against Fall armyworm (*Spodoptera frugiperda*). *J Life Sci Biomed*, 2023; 13(1): 17-24. DOI: https://dx.doi.org/10.54203/jlsb.2023.3

Table 1. General Linear Model: Univariate Analysis of Variance for FAW larvae mortality rate for tested days, Zoolocumulative mortality rate of FAW larvae as dependent variable

Days	Source	DF	Sum of Square	Mean Square	F-Ratio	P-Value
1st	Treatments	5	356.94	71.39		
	Error	12	400.00	33.33	2.14	0.130
	Total	17	756.94			
2nd	Treatments	5	8694.4	1738.9		
	Error	12	2016.7	168.1	10.35	0.001*
	Total	17	10711.1			
3rd	Treatments	5	256.94	51.39		
	Error	12	600.00	50.00	1.03	0.444
	Total	17	856.94			
4th	Treatments	5	361.11	72.22		
	Error	12	916.67	76.39	0.95	0.487
	Total	17	1277.78			
5th	Treatments	5	344.44	68.89		
	Error	12	983.33	81.94	0.84	0.546
	Total	17	1317.77			
6th	Treatments	5	327.78	65.56		
	Error	12	483.33	40.28	1.63	0.227
	Total	17	811.11			
7th	Treatments	5	540.28	108.06		
	Error	12	616.67	51.39	2.10	0.135
	Total	17	1156.94			
8th	Treatments	5	90.28	18.06		
	Error	12	166.67	13.89	1.30	0.327
	Total	17	256.94			
Cumulative mortality rate among treatments	Treatments	5	16261.1	3252.2		
	Error	12	683.33	56.94	57.11	0.0001*
	Total	17	16944.4			

*Significance P<0.0001 for mortality monitored days and cumulative mortality

Table 2. Post-hoc tests of treatments of second day mortality rate of FAW larvae using Tukey's HSD tests at 95.0% confidence interval.

Treatments	Mean mortality (%± SE)	95% CI (lower, upper)
F1	46.67 ± 10.58^{b}	(36.09, 57.25)
F2	10.0 ± 10.58^{ac}	(-0.58, 20.58)
F3	25.0 ± 10.58^{bc}	(14.42, 35.58)
F4	65.0 ± 10.58^{b}	(54.42, 75.58)
F5	20.0 ± 10.58^{abc}	(9.42, 30.58)
Control (-)	0.0 ± 10.58^{a}	(-10.58, 10.58)

*Means followed by different letters with in a column are significantly different at α = 0.05.

Table 3. Post-hoc tests of cumulative mortality rate of FAW larvae for the treatments using Tukey's HSD tests at 95.0% confidence interval.

Treatments	Mean mortality (%± SE)	95% CI (lower, upper)
F1	96.67 ± 6.16^{b}	(90.51, 100.0)
F2	86.66 ± 6.16^{bc}	(80.50, 92.82)
F3	83.34 ± 6.16^{bc}	(77.18, 89.50)
F4	91.66 ± 6.16^{bc}	(85.50, 97.82)
F5	80.00 ± 6.1^{6c}	(73.84, 86.16)
Control (-)	8.33 ± 6.16^{a}	(2.17, 14.49)

*Means followed by different letters with in a column are significant difference at α = 0.05.

CONCLUSIONS

The use of entomopathogenic fungi is one of the environmentally friendly strategies for reducing the effects of the fall armyworm. To control *S. frugiperda*, it is possible to isolate potential entomopathogenic fungi from locally accessible soil. Entomopathogenic fungi infect the host insect and cause disease due to the fact that fungi are widely distributed in the soil environment. Isolating and using effective entomopathogenic fungi from pest host ecology to control insect pest populations is environmentally friendly. According to our research, isolated fungi can result in fall armyworm larval mortality rates of 80.0 to 96.67%. This finding supports the ongoing search for a biological fall armyworm control strategy. Naturally, more research is needed on their conidial concentration, efficacy, and effectiveness in the field, as well as on their characterization and species-level identification.

DECLARATIONS

Corresponding author
E-mail: aberaabos@gmail.com; ORCID: 000-0003-3385-6369

Authors' contribution

Abera HD had the idea for the research design, performed the literature search, conceived and designed the experiments; performed data collection; analyzed and interpreted the data; wrote the first draft, revised and formatted the manuscript. Emana GD made substantial and technical contributions to the research in supervising and the structure of the manuscript drafts. Both authors participated in the design of study, reviewed and approved the final manuscript.

Acknowledgements

The authors thank the staff of Plant protection laboratory of Melkassa agricultural research center (MARC) for their help to conduct the experiment and Dr. Tesfay Alemu and w/ro Zenebech for their support at mycology laboratory of Addis Ababa University (AAU).

Funding support

None.

Competing interests

The authors declare that they have no competing interests.

Ethical approval

The review board and ethics committee of Department of Zoological sciences, AAU approved. All methods were performed in accordance with the relevant guidelines and regulations.

REFERENCES

[1] Food and Agricultural Organization of the United Nations (FAO) 2019. Briefing note on FAO actions on fall armyworm. http://www.fao.org/3/BS183E/bs183e.pdf

[2] Ferreira FJBS, Alves L, Gottardo L, Georgino M Dimension amentodo custoeconomico represent adop or Spodopterafrugiperdanacultura do milho no Brasil. 48 Congresso Sociedade Brasileira de Economia, Administracão e Sociologia Rural, 21. [Economic cost dimensioning represents adopting or Spodoptera Frugiperda Maize crop in Brazil. 48 Congress Brazilian Society of Economics, Administration and Rural Sociology, 21]. 2010.

[3] Food and Agricultural Organization of the United Nations (FAO). Integrated management of the Fall Armyworm on maize. A guide for Farmer Field Schools in Africa. 2018. https://reliefweb.int/report/world/integrated-management-fall-armyworm-maize-guide-farmer-field-schools-africa

[4] International Institute of Tropical Agriculture (IITA) First report of outbreaks of the "Fall Armyworm" on the African continent. IITA Bulletin, No. 2330. 2016. http://bulletin.iita.org/index.php/first-report- of out breaks-of-the-fall-armyworm-on-the-africancontinent/

[5] International Plant Protection Convention (IPPC) The damage caused by *Spodoptera frugiperda*. IPPC Official Pest Report. Rome, Italy: FAO. 2016. https://www.ippc.int/

[6] Cock MJW, Beseh BK, Buddie AG, Cafa G, Crozier J, Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. Scientific Reports, 2017; 7: 4103. DOI: https://doi.org/10.1038/s41598-017-04238-y
[7] ACAPS Thematic Report: Fall Armyworm outbreak in Africa. 2017. http://reliefweb.int/sites/reliefweb.int/files/resources/
[8] Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, Subramanian S, Tefera T. Fall Armyworm, Spodopterafrugiperda Infestations in East Africa: Assessment of Damage and Parasitism. *Insects*, 2019; 10:195. DOI: https://doi.org/10.3390/insects10070195
[9] Hruska AJ. Fall armyworm (*Spodoptera frugiperda*) management by smallholders. *CAB reviews*, 2019; 14(043). DOI: https://doi.org/10.1079/PAVSNNR201914043
[10] Du Plessis H, van den Berg J, Kriticos O *Spodopterafrugiperda*. CSIRO-InSTePP*Pest Geography*. Canberra. 2018.
[11] Early R, Gonzalez-Moreno P, Murphy ST, Day R. Forecasting the global extent of invasion of the cereal pest *Spodopterafrugiperda*, the fall armyworm. *Neo Biota*., 2018; 40: 25-50. DOI: https://doi.org/10.3897/neobiota.40.28165
[12] Tefera T, Goftishu M, Ba M, Muniappan R. A Guide to Biological Control of Fall Armyworm in Africa Using Egg Parasitoids. *First Edition*, Nairobi, Kenya. 2019; http://hdl.handle.net/123456789/1001
[13] Montezano DG, Specht A, Sosa-Gómez DR, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SVD, Peterson JA, Hunt TE. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *African Entomol.*, 2018; 26: 286 -301. DOI:https://doi.org/10.4001/003.026.0286
[14] Luginbill P 1928. The Fall Armyworm. Technical bulletin No. 34. United States Department of Agriculture Washington, D.C., pp. 91 (E-book). https://naldc.nal.usda.gov/download/CAT90913266/PDF
[15] Abrahams P, Bateman M, Beale T, Clottey V, Cock M, Colmenarez Y, et al. Fall armyworm: Impacts and Implications for Africa; Evidence Note (2); CABI: Oxford shire, UK. 2017; DOI: https://doi.org/10.1564/v28_oct_02
[16] Baudron F, Zaman-Allah MA, Chaipa I, Chari N, Chinwada P. Understanding the factors conditioning fall armyworm (*Spodopterafrugiperda* J.E. Smith) infestation in African smallholder maize fields and quantifying its impact on yield: a case study in Eastern Zimbabwe. Crop Protect., 2019; 120:141-150. DOI: https://doi.org/10.1016/j.cropro.2019.01.028
[17] Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M, et al. A Fall armyworm: impacts and implications for Africa. Outlooks Pest management, 2017; 28: 196-201. DOI: https://doi.org/10.1564/v28.
[18] Kumela T, Simiyu J, SisayB, Likhayo P, MendesilE, Gohole L, TeferaT Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodopterafrugiperda*) in Ethiopia and Kenya. *Int, J. Pest Manage.* 2018; 0874: 1-9. DOI: https://doi.org/10.1080/09670874.2017.1423129
[19] Midingoyi SKG, Menale Kassie, Muriithi B, Diiro G andEkesi S Do farmers and the environment benefit from adopting integrated pest management practices? Evidence from Kenya. J. Agri. Econ., 2018; 70(2): 452-470. DOI: https://doi.org/10.1111/1477-9552.12306.
[20] Yu SJ. Insecticide resistance in the fall armyworm, *Spodopterafrugiperda* (JE Smith). *Pestic. Biochem. Phys*., 1991; 39: 84-91. DOI:https://doi.org/10.1016/0048-3575
[21] Ríos-Velasco C, Cerna-Chávez E, Sánchez-Peña S, Gallegos-Morales G. Natural epizootic of the entomopathogenic fungus Nomuraearileyi (Farlow) Samson infecting *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Coahuila México. *J. Res. Lepid*. 2010; 43: 7-8.
[22] Molina-Ochoa J, Lezama-Gutiérrez R, GonzálezRamírez M, López-Edwards M, Rodríguez-Vega MA, Arceo-Palacios F. Pathogens and parasitic nematodes associated with populations of fall armyworm (Lepidoptera: Noctuidae) larvae in Mexico. *Fla Entomol*. 2003; 86: 244-253. DOI: https://doi.org/10.1653/0015-4040
[23] Lezama-Gutiérrez R, Alatorre-Rosas R, Bojalil-Jaber LF., Molina-Ochoa J, Arenas-Vargas M, González-Ramírez M, Rebolledo-Domínguez O. Virulence of five entomopathogenic fungi (Hyphomycetes) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs and neonate larvae. Vedalia, 1996; 3: 35-39.
[24] Eilenberg J, Hajek A, Lomer C. Suggestions for unifying the terminology in biological control. *Biol. Control*. 2001; 46: 387-400. DOI: https://doi.org/10.1023/A:1014193329979
[25] Shah PA, Pell JK. Entomopathogenic fungi as biological control agents. *Appl. Microbiol. Biotechnol*. 2003; 61: 413-423. DOI: https://doi.org/10.1007/s00253-003-1240-8
[26] Ruiu SA, Floris I. Emerging entomopathogenic bacteria for insect pest management. *Bulletin of Insectology*. 2013; 66 (2): 181-186.ISSN1721-8861
[27] Maistrou S, Natsopoulou ME, Jensen AB, Meyling NV. Virulence traits within a community of the fungal entomopathogen Beauveria: Associations with abundance and distribution. *Fungal Ecol.* 2020; 48:100992. DOI:https://doi.org/10.1016/j.funeco.2020.100992
[28] Litwin A, Nowak M, Rozalska S. Entomopathogenic fungi: unconventional applications. *Rev Environ Sci. Biotechnol.* 2020; 19 (1): 23-42. DOI: https://doi.org/10.1007/s11157-020-09525-1
[29] Shahzad MA, Irfan M, Wahab AA, Zafar F, Abdulrehman, et al. 2021. Toxicity of entomopathogenic fungi against *Spodopterafrugiperda* larvae under laboratory conditions. *J. Agric. Sci. Food Technol.*, 7(3):355-358. DOI: https://doi.org/10.17352/2455-815X.000131
[30] Rajula J, Pittarate S, Suwannarach N, Kumla J PtaszynskaAAThungrabeab M, Mekchay S, Krutmuang P. Evaluation of Native Entomopathogenic Fungi for the Control of Fall Armyworm (*Spodopterafrugiperda*) in Thailand: A Sustainable Way for Eco-Friendly Agriculture. *J. Fungi*, 2021; 7: 1073. DOI.https://doi.org/10.3390/jof7121073

[31] Akutse KS, Kimemia JW, Ekesi S, Khamis FM, Ombura OL, Subramanian S. Ovicidal effects of entomopathogenic fungal isolates on the invasive fallarmyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Appl. Entomol.*, 2019; DOI: https://doi.org/10.1111/jen.12634

[32] Ramanujam B Poornesha B, Shylesha AN. Effect of entomopathogenic fungi against invasive pest *Spodopterafrugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize. Egypt. *J. Biol. Pest Control*. 2020; 30: 1-5. DOI: https://doi.org/10.1186/s41938-020-00291-4

[33] Ramirez-Rodriguez D, Sánchez-Peña SR. EndophyticBeauveriabassiana 1 in zea mays: Pathogenicity against larvae of fall armyworm, *Spodoptera frugiperda* Southwest. *Entomol.,* 2016; 41: 875–878. DOI:https://doi.org/10.3958/059.041.0330

[34] Idrees A, Qadir ZA, Akutse KS, Afzal A, Hussain M, Islam W, et al. Effectiveness of Entomopathogenic Fungi on Immature Stages and Feeding Performance of Fall Armyworm, *Spodopterafrugiperda* (Lepidoptera: Noctuidae) Larvae. *Insects*, 2021; 12: 1044. DOI: https://doi.org/10.3390/insects12111044

[35] Pitre H, Hogg D. Development of the fall armyworm on cotton, soybean and corn (*Spodopterafrugiperda*). *J. of the Georgia Entomol. Society*. ISSN 0016-8238; 1983.

[36] Cruz-Avalos AM, Bivián-Hernández MÁ, Ibarra JE, Rincón-Castro Ma CD. High Virulence of Mexican Entomopathogenic Fungi against Fall Armyworm, (Lepidoptera: Noctuidae). *J. Economic Entomol.*, 2019; 112(1): 99-107. DOI: https://doi.org/10.1093/jee/toy343

[37] Hassan FR, Abdullah SK, Assaf LH. Pathogenicity of the entomopathogenic fungus, *Beauveriabassiana* (Bals.) Vuill. endophytic and a soil isolate against the squash beetle, *Epilachnachrysomelina* (F.) (Coleoptera: Coccinellidae). *Egypt. J. Biol. Pest Control*, 2019; 29: 1-7. DOI: https://doi.org/10.1186/s41938-019-0169-x

[38] World Health organization (WHO). Tests procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Report of the WHO Informal Consultation, Geneva, Switzerland, 1998; Pp. 43. https://apps.who.int/iris/handle/10665/64879