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RESEARCH ARTICLE

PESTICIDAL ACTIVITY OF CRUDE EXTRACTS OF ALOE SECUNDIFLORA,NICOTIANA TABACUM AND TEPROSIA VOGELII AGAINST FALL ARMYWORM (SPODOPTERA FRUGIPERDA)

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Abstract

The fall armyworm (FAW),Spodoptera frugiperda (Lepidoptera: Noctuidae) is a recent invasive pest species that has successfully established across sub-Saharan Africa. Management of FAW in its native range in the Americas has led to the development of resistance to many commercial pesticides before its arrival in Africa. Synthetic pesticides are often associated with issues such as pest resistance, persistent residue, non-target toxicity, and environmental issues. Therefore, the research and development of novel, safe, and effective pesticides has become a focus in pesticide discovery. Pesticide use may therefore be ineffective for FAW control in Africa, so new and more sustainable approaches to pest management are required that can help reduce the impact of FAW pest. Pesticidal plants provide an effective and established approach to pest management in African smallholder farming and recent research has shown that their use can be cost-beneficial and sustainable. In this study, crude leave extracts of three plants aloe secundiflora, nicotiana tabacum and teprosia vogelii were evaluated against fall armyworm (spodoptera frugiperda). Two different concentrations, 30mg/L and 15mg/L were evaluated in this study against second instar FAW larvae and eggs. The results indicated that these leave extracts caused a larval and egg mortalities of different levels. On average leave extracts of aloe secundiflora, teprosia vogelii and nicotiana tabacum caused larval mortalities of 80%, 63.33% and 93.33% respectively for 30mg/L concentrations while the average larval mortalities for aloe secundiflora, teprosia vogelii and nicotiana tabacum were 26.67%, 33.33% and 80.0% respectively for the 15mg/L concentrations. The egg mortalities were relatively low compared to larval mortalities for the two concentrations used in this study. On average the leave extracts of aloe secundiflora, teprosia vogelii and nicotiana tabacum caused the egg mortalities of 16.67%, 13.33% and 43.33% respectively when 30mg/L were used while the average egg mortalities for aloe secundiflora, teprosia vogelii

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and nicotiana tabacum were 3.33%, 0.0% and 26.67% respectively for the 15mg/L concentrations. It follows that nicotiana tabacum showed high larval and egg mortalities for both concentrations compared to the other two leave extracts and therefore good pesticide for the control FAW larvae and eggs.

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Introduction:-

The fall armyworm (FAW) is a highly polyphagous insect pest that attacks more than 80 plant species, including maize, sorghum, millet, sugarcane, and vegetable crops; nevertheless, maize is the main crop affected by FAW in Africa. Given the importance of maize in Africa as a primary staple food crop, the recent invasion of FAW threatens the food security of millions of people in a region (Sisay et al., 2019). The invasive FAW, Spodoptera frugiperda, has been one of the most rapidly spreading and highly devastating maize pests across Africa and Asia (Tambo et al., 2021). Since the occurrence of FAW in African countries, synthetic insecticides have been widely used as an emergency response to slow the spread of the pest and minimize damage to maize fields. Although synthetic insecticides play an important role in FAW management, given confirmed reports of the development of insecticide resistance in FAW populations as well as other adverse effects due to the sole dependence on synthetic insecticide, it is imperative to use an integrated pest management strategy for FAW (Yu, 1991).

The overuse of synthetic insecticides to eradicate this pest is manifested as environmental contamination and insecticidal resistance in S. frugiperda. Approximately 46% and 60% of farmers in Ethiopia and Kenya, respectively, claimed the ineffectiveness of synthetic insecticides against S. frugiperda. Indeed, repeated applications of insecticides with the same mode of action have resulted in resistance to S. frugiperda in Africa. Furthermore, Zhang et al. monitored the resistance in S. frugiperda against commonly used insecticides and revealed the evolution of resistance in S. frugiperda against chlorpyrifos, spinosad, lambda-cyhalothrin, malathion, fenvalerate, deltamethrin, emamectin benzoate and chlorantraniliprole (Ahmed et al., 2022)

While pests in Africa continue to limit food crop harvests, studies indicate that losses due to pests overall are in the region of 30 % (Grzywacz et al., 2014), but localized losses due to outbreaks of major migratory pests such as locusts and armyworms can be even greater, sometimes resulting in complete crop failure (Rose et al., 2000). While crop pests are a problem in all cropping systems globally, their impact is much greater in sub-Saharan Africa, where poverty, limited knowledge and poor agricultural infrastructure mean that much subsistence farming is conducted without access to effective crop protection knowledge or resources (Grzywacz et al., 2014).

A recent study by Abrahams et al. (2017) has shown that in the absence of control methods, FAW has potential to cause about 21% to 53% reduction in annual maize production (or US$2,481 to US$6,187 million economic damage) in 12 maize-producing countries in Africa. Considering that the pest cannot be eradicated (FAO 2018), implementation of control methods is, therefore, critical in curbing these potential negative economic impacts of FAW in Africa (Tambo et al., 2020). Farmers have complained that the currently used synthetic insecticides are not effective against FAW; hence, they are forced to use high doses with frequent applications, which will lead to the accumulation of pesticides in the environment and speed up resistance development (Sisay et al., 2019).

As chemical pesticides are withdrawn owing to resistance problems or because they are no longer commercially viable, opportunities are emerging for biorational solutions. Biopesticides offer a more sustainable solution to pest control than synthetic alternatives and do not feature residue problems, which are a matter of significant concern for consumers. Although biopesticides are not as effective as chemical their compatibility with synthetic pesticides varies, and shelf life is often shorter (Gonzalez-Coloma et al., 2010). This therefore makes this study to consider biopesticide for the control of FAW.

Since past many years till today pests control in crop production is largely dependent on synthetic pesticides which have worsen the environment as well as many pesticide resistant reports in crops have been emerged. Biopesticides certainly offer a better way to manage plant diseases and environment simultaneously. Biopesticides have tremendous potential to replace or reduce synthetic pesticides usage and cost incurred in them

In the absence of effective alternative management options to tackle pests, smallholder farmers rely extensively on indiscriminate application of synthetic pesticides. These synthetic pesticides are harmful to human health, detrimental to the environment and biodiversity, and lead to rapid build-up of resistance in the target pests while decimating natural enemies of pests, resulting in secondary pest outbreaks. In addition, presence of pesticide residues on export crops that are above the permissible maximum residue levels of importing nations results in informal trade barriers (Bailey et al., 2010).

Botanical extracts have long been proposed as attractive alternatives to synthetic insecticides for pest management. Botanical extracts are eco-friendly, economical, usually target-specific, and biodegradable. The greatest strength of botanical extracts is their specificity, as most are essentially nontoxic and non-pathogenic to animals and humans (Ofori et al., 2020); (Stevenson et al., 2012). Various plant species have shown insecticidal properties against FAW, for example extracts of neem, Azadirachtaindica (Silva et al., 2015). Botanicals are cheap, readily available, and affordable, which are important qualities of pest control products for smallholder farmers in Africa (Sisay et al., 2019).

Studies and development of biospesticides for control of FAW will be of great importance since it will offer farmers safe and environmentally friendly pest management practices. ICIPE and its partners are in the process of developing biopesticides targeting Africa's invasive and migrant pest particularly s. frugiperda and desert locust schistocerca gregaria. The fact that in Africa, biopesticide use is still at its infancy and only accounts for 3% of the world biopesticide market calls for more research focusing on biopesticide development.

Biopesticides are considered to be the best alternative to synthetic pesticides that are highly effective, target specific and reduce environmental risks. The advance research and development in the field of biopesticide applications greatly reduces the environmental pollution caused by the chemically synthetic insecticides residues and promotes sustainable development of agriculture.

The aim of this study was to investigate the pesticidal activity of three plants leave extracts i.e nicotiana tabacum, teprosia vogelii and aloe secundiflora against second instar FAW larvae and eggs.

Nicotiana tabacum

Nicotiana tabacum, or cultivated tobacco, is an annually-grown herbaceous plant. It is found in cultivation, where it is the most commonly grown of all plants in the genus Nicotiana, and its leaves are commercially grown in many countries to be processed into tobacco. All parts of the plant contain nicotine, which can be extracted and used as an insecticide. The dried leaves can also be used; they remain effective for 6 months after drying. The juice of the leaves can be rubbed on the body as an insect repellent. The leaves can be dried and chewed as an intoxicant. The dried leaves are also used as snuff or are smoked. This is the main species that is used to make cigarettes, cigars, and other products for smokers. A drying oil is obtained from the seed (Wennig, 2009). The plant galls are widely distributed, and their extracts are used in traditional medicine worldwide. Traditional remedies containing extracts of plant galls in China, India, and some African countries are effective in the treatment of various pathologies. The leaves are applied externally in the treatment of rheumatic swelling, skin diseases, painful piles, and stings (Agyare et al., 2013).

Teprosia vogelii

It is a herb or small tree that is native to tropical Africa and has also been used in tropical America as well as South and Southeast Asia. It is commonly used to deter pests and diseases, specifically fleas and ticks on animals. It is not suitable for livestock or human consumption because it is not highly nutritious and can be poisonous for fish and some other animals (Azimova & Glushenkova, 2012).

It is a small tree used by farmers in numerous countries in Africa to get rid of pests on livestock, control pests in cultivated fields as an organic pesticide, improves soil fertility, as a medicine for skin diseases and internal worms, and for storage of crops.The use of Tephrosia leaf extract as a low cost acaricide is spreading to farmers in central Kenya and has been very successful in terms of its results (Gadzirayi et al., 2009; Ndava et al., 2018). Crude extract from leaves of Tephrosia vogelii is potentially used to control ticks and worms in the Ugandan animal production systems. It has also been used to control larval stages of mosquitoes and is effective against soft bodied insects and mites including aphids and red spider mites. Dried leaves have the potential to protect stored legume seeds from damage by the bruchids as used by farmers in Southern Africa (Stevenson et al., 2012).

Aloe secundiflora

Aloe secundiflora is an evergreen, succulent, perennial plant that produces a dense rosette of about 20 spear-shaped leaves that can be 30 - 75cm long and 8 - 30cm wide at the base. The plant can be stemless, or with a short stem up to 30cm long; it is usually solitary, but sometimes suckers to form small groups.The plant is harvested from the wild for local use as a medicine and fermenting agent. The plant is harvested from the wild in Kenya on a commercial scale to obtain the exudate known as 'bitters' from its leaves (http://www.prota.org).

The leaf sap is drunk as an appetizer and anti-emetic. Diluted leaf sap is drunk as a cure for malaria, typhoid fever, diarrhoea, oedema, swollen diaphragm, nosebleed, headache, pneumonia, chest pain and as a disinfectant. The leaves are applied to wounds to assist healing. The exudate is applied into the eyes, to cure conjunctivitis. The bitter exudate is applied to nipples to wean children (http://www.prota.org). Aloe secundiflora leaf components have been credited for antibacterial, antifungal and antiviral and antihelmintic medicinal properties. Aloe products have also been used in pharmaceuticals, cosmetic and food industries (Kaingu et al., 2012).

Experimental methods and materials

Study Area

The study area was Elgeyo Marakwet County, Keiyo North sub-county. Nicotiana tabacum and Aloe secundiflora leave samples were obtained from Tambach ward of Keiyo north Sub-county while Teprosia vogelii leave samples were collected from Kamariny ward of Keiyo north sub-county.

Collection, Identification and Extraction of plant material

Fresh plant leaves from each plant species were collected from the identified sampling areas and identified by a plant taxonomist at the Department of Biological Sciences, School of Pure and Applied Sciences of Mount Kenya University.

The leaves were cleaned using distilled water and oven dried at 40°C for 72 hrs, ground in a mill. Exactly 300 g of ground plant material from each plant species was extracted for 24 h in 70% ethanol (ethanol:water, 7:3 v/v) by maceration with agitation. The sample was then filtered, and the resulting filtrate concentrated in a rotary evaporator under low pressure at 50°C. The filtrate obtained was stored on glass bottles awaiting bioassay (dos Santos et al., 2016).

Fall Armyworm Rearing and maintenance

FAW larvae were collected from maize fields in Kiambu county, Thika town sub-county, Thika Township ward. To establish a large colony the larvae are initially reared on portions of young maize leaves; and later on the larvae was reared on an artificial diet. The artificial diet composed of maize leaf powder, common bean powder, brewer's yeast, ascorbic acid, sorbic acid, methyl-p-hydroxybenzoate, vitamin E capsules, sucrose, formaldehyde and agar (Prasanna et al., 2018).

Neonates and 2nd instars were reared in 500 mL plastic containers containing young maize leaves, which were renewed daily. At the 2nd instar, the larvae were transferred to individual plastic containers (100 mL) to reduce cannibalism until pre-pupal stage and fed on an artificial diet that is changed every week until pupation. The pupae were harvested and transferred in Petri dishes lined with tissue paper and placed in adult emergence cages (mosquito netting around an 18 x 18 x 18 cm frame). After adult emergence, the moths were fed on honey from a honey-soaked wad of cotton wool in a container placed in each cage. Eggs laid on filter paper in the cages were removed daily and are disinfected by dipping them in 10% formaldehyde for 15 min. The eggs were rinsed thoroughly with distilled water and dried on filter paper. Thereafter, eggs were placed on 100mL containers until they hatch to repeat the rearing process (Prasanna et al., 2018).

Bioassay on FAW larvae

The efficacy of the leave extracts at 30 mg/L were evaluated on second instar larvae of S. frugiperda. Bioassay was performed on Petri dishes (5.0 cm diameter x 1.2 cm height) containing the leave extract, the quantity was determined in preliminary tests. Five petri dishes were used for each plant extract, three dishes contained 10 FAW larvae having treated fresh maize leaves, one positive control and one negative control. The positive control contained fresh maize leaves treated with a synthetic pesticide (1ml/L of Dudutrin) while the negative control contained untreated fresh maize leaves. In each petri dish 10 FAW larvae were placed and allowed to feed on the

leaves.The FAW larvae were exposed to the extracts for 24 hours by allowing the larvae feed on treated fresh leaves which were replaced after every 48 hours. Testing was repeated using 15 mg/L of the leave extracts.

Subsequently, the numbers of live and dead larvae were counted after 24 hours for five days.

Bioassay on egg hatch

The efficacy of leave extracts at 30 mg/L were evaluated on FAW eggs. Bioassay were done on petri dishes. Five petri dishes were used each having 10 FAW eggs. The FAW eggs in three petri dishes were sprayed with 30mg/L of the plant extract while for positive control dish it was sprayed with the synthetic pesticide (dudutrin) and the negative control the FAW eggs were sprayed with distilled water. Spraying was repeated after every 48 hours. Testing was repeated using 15 mg/L of the leave extracts.

Subsequently, the numbers of hatched and unhatched FAW eggs were counted after 24 hours. The counting was repeated after every 24 hours until the fifth day.

Results and Discussions:-

Results for 30mg/L leave extracts

The results for the bioassays using 30mg/L leave extracts against FAW larvae are tabulated for each leave extract in the tables below.

Table 1:- Aloe secundiflora.

Duration (hours)	Replicate Expts (Cummulative % mortality)				
	ALV 1	ALV 2	ALV 3	–VE CTRL	+VE CTRL
24 hours	40	30	0	0	100
48 hours	70	50	10	0	NA
72 hours	80	70	30	0	NA
96 hours	80	80	60	0	NA
120 hours	80	80	80	10	NA

The three replicate extracts of Aloe secundiflora i.e ALV 1, ALV 2 & ALV 3 were able to cause on average mortality of 80% on the FAW larvae within 120 hours (Table 1). The +VE control killed all the FAW larvae within 24 hrs while in the –VE control 10% FAW larvae died naturally.

Table 2:- Teprosia vogelii.

Duration (hours)	Replicate Expts (Cummulative % mortality)				
	TEV 1	TEV 2	TEV 3	–VE CTRL	+VE CTRL
24 hours	10	60	20	0	100
48 hours	30	70	40	0	NA
72 hours	40	80	40	0	NA
96 hours	50	90	50	0	NA
120 hours	50	90	50	20	NA

TEV 1 & TEV 3 were able to kill 50% of the FAW larvae within 5 days. TEV 2 was able to cause a mortality of 90% of the FAW larvae within 120 hours (Table 2). The +VE control was able to kill 100% of FAW larvae while the -VE control 20% died within 120 hours.

Table 3:- Nicotiana tabacum.

Duration (hours)	Replicate Expts (Cummulative % mortality)				
	NIT 1	NIT 2	NIT 3	–VE CTRL	+VE CTRL
24 hours	40	30	30	0	100
48 hours	80	60	30	0	NA
72 hours	90	90	90	0	NA
96 hours	90	100	90	0	NA
120 hours	90	100	90	20	NA

NIT 1 & NIT 3 were able to kill 90% of the FAW larvae within 120 hours. NIT 2 was able to kill all the FAW larvae within 120 hours (Table 3). The +VE control was able to cause 100% mortality of FAW larvae within 24 hours

while in the –VE control 20% of FAW larvae died within the 120 hours. Nicotiana tabacum leave extracts were able to cause the highest mortality among the three leave extracts used in this study.

Results for 15mg/L leave extracts

The results for the bioassay using 15mg/L leave extracts against FAW larvae are tabulated for each leave extract in the tables below.

Table 4:- Aloe secundiflora.

Duration (hours)	Replicate Expts (Cummulative % mortality)				
	ALV 1	ALV 2	ALV 3	–VE CTRL	+VE CTRL
24 hours	0	0	0	0	100
48 hours	10	0	10	0	NA
72 hours	10	0	20	0	NA
96 hours	20	0	30	0	NA
120 hours	30	0	30	0	NA
144 hours	30	10	30	0	NA
168 hours	30	20	30	0	NA

Aloe secundiflora extracts were able to cause on average 26.67% mortality of FAW larvae (Table 4). A. secundiflora reported the lowest mortality among the three leave extracts.

Table 5:- Teprosia vogelii.

Duration (hours)	Replicate Expts (Cummulative % mortality)				
	TEV 1	TEV 2	TEV 3	–VE CTRL	+VE CTRL
24 hours	0	0	0	0	100
48 hours	0	0	10	0	100
72 hours	0	10	10	0	100
96 hours	0	10	10	0	100
120 hours	10	20	30	0	100
144 hours	30	20	50	0	100
168 hours	30	20	50	0	100

Teprosia vogelii leave extracts were able to cause on average 33.33% mortality of FAW larvae. TEV 2 reported the lowest mortality of 20% while TEV 3 reported the highest mortality of 50% (Table 5).

Table 6:- Nicotiana tabacum.

Duration (hours)	Replicate Expts (Cummulative % mortality)				
	NIT 1	NIT 2	NIT 3	–VE CTRL	+VE CTRL
24 hours	10	10	10	0	100
48 hours	20	20	20	0	100
72 hours	50	30	20	0	100
96 hours	50	60	30	0	100
120 hours	70	70	40	0	100
144 hours	90	70	50	0	100
168 hours	100	90	50	0	100

On average Nicotiana tabacum leave extracts caused a mortality of 80% on FAW larvae. NIT 3 reported the lowest mortality of 50% while NIT 1 reported the highest mortality of 100% implying that all the 10 larvae died within the 168 hours of exposure (Table 6). Nicotiana tabacum reported the highest mortality among the three leave extracts.

Bioassay of FAW eggs

Table 7:- Number of hatched FAW eggs - using 15mg/L leave extracts.

Extract	Replicates	Day 1 -4	Day 5	Day 6	Day 7	No. of hatched eggs (%)	Average No. of hatched eggs (%)
NIT	1	0	6	0	0	60	73.33
	2	0	8	1	0	90	

	3	0	6	1	0	70	
ALV	1	0	10	N/A	N/A	100	96.67
	2	0	10	N/A	N/A	100	
	3	0	9	0	0	90	
TEV	1	0	10	N/A	N/A	100	100
	2	0	10	N/A	N/A	100	
	3	0	9	1	0	100	
+VE CTRL		0	0	0	0	0	0
-VE CTRL		0	9	1	N/A	100	100

Table 8:- Number of hatched FAW eggs - using 30mg/L leave extracts.

Extract	Replicates	Day 1 -4	Day 5	Day 6	Day 7	No. of hatched eggs (%)	Average No. of hatched eggs (%)
NIT	1	0	0	7	0	70	56.67
	2	0	0	2	3	50	
	3	0	0	3	2	50	
ALV	1	0	7	0	0	70	83.33
	2	0	10	N/A	N/A	100	
	3	0	7	0	1	80	
TEV	1	0	0	9	0	90	86.67
	2	0	0	5	3	80	
	3	0	0	5	4	90	
+VE CTRL		0	0	0	0	0	0
-VE CTRL		0	8	2	N/A	100	100

N.tabacum showed the highest mortality for both 30mg/L (56.67% eggs hatched) and 15mg/L(73.33% eggs hatched) compared to T. vogelii using 30mg/L (86.67% eggs hatched) while using 15mg/L (100% eggs hatched). For A. secundiflora using 30mg/L 83.33% eggs hatched while using 15mg/L 96.67% eggs hatched. 30mg/L extracts generally showed higher mortalities compared to 15mg/L (Table 7 and 8).

On average N. tabacum reported the highest mortality followed by A. secundiflora while T. vogelii reported the lowest mortality against FAW eggs for both 15mg/L and 30mg/L.

Conclusion:-

The study found that the three leave extracts from the three plants i.e aloe secundiflora, nicotiana tabacum and teprosia vogelii showed antifeedant activity against fall armyworms larvae though to different levels. N. tabacum showed the highest mortality on FAW larvae while T. vogelii showed the lowest mortality. Despite considerable work on its biopesticidal effects, Nicotiana tabacum is arguably the plant species with the highest vertebrate toxicity, well-known for the effects of nicotine and related alkaloids. However, despite potential human toxicity dangers, N. tabacum is being pursued as one of several potential botanical options for FAW control in several African countries, and thus merits further investigation regarding its safe use and non-target effect. From the study it is evident that management of larvae using these leave extracts is comparatively easier compared with the eggs.

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