The efficacy of Tephrosia vogelii and Azadirachta indica leaf extract combinations against fall Armyworm, Spodoptera frugiperda, in Maize.

James Nemeyimana (jamesnemeyimana@gmail.com)
Mbarara University of Science and Technology
Rashid S Muhooozi
Mbarara University of Science and Technology
Upton Nuwagira
Mbarara University of Science and Technology
Francis Ssenkuba
Mbarara University of Science and Technology

Research Article

Keywords:

Posted Date: August 28th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3237815/v1

Abstract

Spodoptera frugiperda is an economical pest affecting Uganda's agriculture. These pests are voracious feeders, polyphagous in nature and mostly affect the cereal crops such as maize and wheat which is the staple food of Africa and Uganda in particular, thus pause a threat to Africa's food security. Ugandan farmers largely depend on synthetic pesticide to control FAW, however these have negative impacts on the environment such as water pollution, death of beneficial organisms such as bees, and development of pesticide resistance, and are toxic to human beings and the aquatic life. The aim of this study was to evaluate the bioactivity of *Tephrosia vogelii* and *Azadirachta indica* leaf extracts as botanicals against *Spodoptera frugiperda* under laboratory conditions. The selected botanicals were subjected to phytochemical screening to examine presence of pesticide phytochemical groups of namely; flavonoids, saponnins, terpenoids, alkaloids, tannins and glycosides as highlighted by different literature Fourth instar larvae were collected from established fields in south western Uganda where there was no application of pesticides before and then n transferred to the biology laboratory of Mbarara University of Science and Technology under the faculty of science where they were reared to obtain enough study populations(second instar generation). Aqueous plant extracts were applied in varying combinations as treatments, the percentage mortality rates were recorded at 12 hours interval for 48 hours after treatment. Sterile tap water and cypermethrin were used as negative and positive controls respectively. The tested treatments of the botanicals were mixed in a combination of *T.vogelii* & *A. indica* powder extract weight per volume respectively, where treatment A(20g:10g), B(10g:20g), C(15g:15g), D(30g *T.vogelii* only) & E(30g *A. indica* only) and a positive control of cypermethrin. Results showed that treatments A, D and the positive control had a higher percentage mortality (> 50%, $p > 0.05$) after 48hours of application compared to treatments B, C and E that had a lower percentage mortality below (< 50%, $p < 0.05$). The botanical treatments that showed higher mortality percentage against FAW larvae contained more of the *T.vogelii* extract, this can be used as an extract in most of the botanicals against the FAW larvae. As an alternative, smallholder farmers can include botanicals in their integrated pest management systems around Uganda and East Africa at large conditions in Ethiopia and elsewhere in Africa.

Introduction

Spodoptera frugiperda (J.E.Smith), also known as the "fall army worm(FAW)," belongs to the Noctuidea family of owlet worms in the Lepidoptera order (Gobernment of Wetern Australia, 2020) FAW undergoes total metamorphosis. The egg is dome-shaped, measuring roughly 0.4 mm in diameter and 0.3 mm in height, with a mass that ranges from 100 to 200 (Bhusal & Chapagain, 2020). Total egg production per female averages about 1500 with a high of over 2000, and the egg stage only lasts two to three days throughout the summer (Bhusal & Chapagain, 2020). The second stage, which has six instars, is the larvae (Bhusal & Chapagain, 2020), the most harmful to host plants (Kumela et al., 2019). Larvae are greenish with a black head in their first instar; by their second instar, the head has turned orange. The body's dorsal surface turns brownish in the third instar, and lateral white lines start to appear. The head is reddish brown and white-marbled in the fourth to sixth instars, and the brownish body has white subdorsal and lateral lines (Bhusal & Chapagain, 2020; Navasero & Navasero, 2020). The brightest part of the day is when larvae tend to hide (Rojas et al., 2004). The larvae stage lasts for around 14 days in the summer and 30 days in the cooler months (Volp & Myron, 2022). Typically, pupation occurs in the earth, 2 to 8 cm below the surface. Reddish brown is the hue of the pupa. The transition from the pupa stage to the adult stage typically takes 20 days (Bhusal & Chapagain, 2020). The adult moths have a lifespan of 15 days on average (Benson, 2017).

In 2016 FAW was first discovered in Africa in the countries of Nigeria and the Democratic Republic of S. Tomé and Principe, (Goergen et al., 2016). It is a pest that is extremely polyphagous and preys on a variety of plant species, including vegetables, sorghum, millet, and maize (Yigezu & Wakgari, 2020). The immature larvae of FAW eat mostly on the epidermal leaf tissue, which results in dead hearts, and make open windows in the leaves, which result in a skeletal structure (Goergen et al., 2016).

Globally, it was predicted that 170,000 ha of India's maize farmland were impacted by FAW, which spread to 10 states across the nation (Sagar et al., 2020). According to Gu and Woo (2019), the Yunnan region of China is the most severely affected by FAW, with 80,000 ha of land affected and major damage to crops like maize, sorghum, sugarcane, and ginger crops. 98.6% of the 11,1992.17 hectares of affected land in China is covered in maize (FAO, 2019). More than 10,000 acres of maize in other provinces, like Cambodia, were impacted by FAW in 2019 (Cambodia News). With an infestation rate ranging from 0.5 to 32%, FAW was found in Bangladesh in 8 regions, 22 districts, and 71 administrative regions.

According to FAO (2019), the expected yield loss from FAW in Thailand ranged from 130 million to 260 million dollars. Given the favorable climatic circumstances in Nepal that allow for the growth of FAW populations, the potential crop loss in maize is estimated to be up to 100% if this pest is not appropriately handled. Similar reports of FAW infestations include about 10,000 ha of land in Indonesia, 16,200 ha of land in Myanmar, and 46,000 ha of land in Vietnam (et al., 2020) (Sagar et al., 2020).

While no steps were taken, numerous African nations, including Ghana and Zambia, suffered losses estimated at 8.5 to 21 million tons, or around 250-630 million US dollars (Sagar et al., 2020). In both Ghana and Zambia, the percentage of maize lost to FAW was estimated to be 45% and 40%, respectively. A total of 30.54 million US dollars' worth of corn was lost by Ethiopia, and the FAW had an impact on around 250,000 ha of agricultural land, or 11% of Kenya's entire maize cultivation area due to FAW attacks, yield losses in Tanzania and Uganda totaled 3.2 million tons and 13.91 million tons, respectively (Sagar et al., 2020).

The use of synthetic pesticides is the main method of controlling FAW, according to a study on small-holder farmers' management techniques against the disease that was carried out in Ghana, Rwanda, Uganda, Zambia, and Zimbabwe. Farmers were not employing protective equipment while handling these pesticides, despite the fact that some of them are exceedingly toxic and are forbidden by agro input authorities (Kenko & Kamta, 2021). According to the World Health Organization (WHO), about 1,000,000 people are affected by acute poisoning by contact with the pesticide(Dad et al., 2022).

The relationship between exposure to synthetic pesticides and human health is thoroughly examined in a survey of the epidemiological literature published between May 2018 and May 2019, starting with agricultural workers, this study found a link between agricultural workers' exposure to synthetic pesticides and poor health outcomes (Jallow et al., 2017). The results are closely tied to various malignancies, genetic damage, oxidative stress, neurological problems, respiratory, metabolic, and thyroid effects, but pesticides in water resources also have a negative impact on human health as well as the health of the overall environment (Ayilara et al., 2023). Because synthetic pesticides are less biodegradable, they have a longer lasting impact on the environment, endangering microbial life and reducing soil respiration by 35%, among other effects. Additionally, they directly exterminate helpful organisms like bees and aquatic organisms (Carvalho, 2017).

In agricultural areas, synthetic pesticides have contaminated about 90% of the water supplies (Wahaab & Badawy, 2004). The bioaccumulation and bio magnification of large amounts of pesticides endangers the aquatic and terrestrial food systems (Carvalho, 2017; Wahaab & Badawy, 2004).According to studies, pesticide exposure is endangering biodiversity and species. In recent decades, European countries have recorded a 70% reduction in insect biomass and a 50% decline in farmland bird (Raven & Wagner, 2021).

Synthetic pesticides have also increased production costs among farmers indicating no significant returns in agriculture as a business, countries on the African continent have increasingly been adopting the use of synthetic pesticides making their import costs higher than before as many don’t have active chemical ingredients that produce synthetic pesticides. The Food and Agriculture Organization of the United Nations (FAO) indicate that in terms of trade, Uganda’s total pesticide import value increased by about 13 times, from US$6.5 to US$82.6 million, between 1994 and 2018 (FAO, 2020), as shown in the figure below(Sustainable & Goals, 2021). With the World Health Organization estimates of about three million cases of acute synthetic pesticide poisoning worldwide and approximately 220,000 death per year (., 2014; Jeyaratnam, 1990; Pandey et al., 2023). It creates an urgent need for research to discover more safe and sustainable approaches for pest control.

Botanical extracts, which are natural extracts derived from pesticidal plants and are either employed as crude extracts or the isolated active components, have the potential to be a safe and sustainable alternative to synthetic pesticides (Samada & Tambunan, 2020). Such botanicals have multiple modes of action, such as repelling, anti-feeding, and anti-oviposition, which makes them less susceptible to pesticide resistance, they are also target specific, less toxic to mammals, and biodegradable as their rate of decomposition increases under solar radiation (Divekar, 2023). Small-holder farmers with limited resources can easily use botanical pesticides because they are affordable, readily available locally, and don't require sophisticated preparation techniques or technology (Grzywacz et al., 2014; Samada & Tambunan, 2020).

To manage field and storage pests, a number of plants, including *Azadirachta indica*, *Phytolaca dodecandria*, *Lantana camara*, *Tithonia diversifolia*, and *Tephrosia vogelii*, have been utilized traditionally in Africa (Samada & Tambunan, 2020). The bioactivity of several botanicals has been tested in studies, and the findings indicate that they are successful in controlling pests in both the field and storage (Samada & Tambunan, 2020).

A. indica and *T. vogelii* are two of the most extensively researched botanical pesticides in the world. In Africa, *A. indica* contains azadirachtin, which has anti-feeding, repellent, anti-oviposition, and anti-molting properties and is effective in controlling over 250 species of pests (Luntz & Nisbet, 2000). Farmers in the Masaka region of Uganda utilize neem leaf extracts to manage field pests including aphids (Julius, 2002; Kamatenesi et al., 2008). According to Asogwa et al. (2010), A. indica leaf and seed powder extracts are efficient against weevils in stored maize, improve seed vigor, and lessen fungal attack and infections on stored seeds.

Traditional names for *T. vogelii* (fish bean) include "Maluku" in Luganda and "Ekiluku" in Runyankore (Julius, 2002). The primary bioactive phytochemicals in *T.vogelii* are rotenoids, which include rotenone, deguelin, and tephrosin. They are highly concentrated in the leaves and have potent insecticidal properties against a variety of pests, as well as anti-feeding and anti-ovipositional properties (Zhang et al., 2020). Furthermore, *Tephrosia vogelli* is heavily promoted in Africa as an agroforestry tree due to its ability to fix nitrogen and as green manure. When grown in Malawi alongside maize, it produced more yield per acre than fertilized monoculture maize (Akinnifesi et al., 2009).

T.vogelli is traditionally used in Malawi and Zimbabwe to protect stored maize and beans against *Sitophilus* ssp (Kamanula et al., 2011). In Zimbabwe, *Tephrosia vogelii* is used to control ticks in dairy animals, a study was done to test its efficacy and it was found that there was no significant difference between T. vogelii and Triatix dip at 5% level (Gadzirayi et al., 2009). It is effective in controlling bruchid beetles (Grzywacz et al., 2014) *T. vogelii* and *A. indica* extracts were tested against *Padagrica* species and investigated in *Hibiscus sabdariffa* in Nigeria, it exhibited high efficacy (59-80%) and a combination of the two was effective as synthetic pesticide delfamethrine (Journal & June, 2013). *T. vogelli* is also effective in controlling field pests in watermelon (*Citrullus lanatus*) (Alao & Adebayo, 2015).

In Africa, Azadirachta indica is also frequently used as a pesticide (Luntz & Nisbet, 2000)with itsmain active component, Azadirachtin, is contained in the seed and tuber . Through a number of different mechanisms of action, azadirachtin is efficient at controlling pests in both the field and storage. According to Ajenifujah-Solebo et al. (2019), it has characteristics that repel, prevent oviposition, and prevent feeding. By preventing the production of morphogenetic peptides and causing sterility by changing ecdysteroid, azadirachtin inhibits molting and decreases the quantity of viable eggs and line progeny (Luntz & Nisbet, 2000).

In Uganda, smallholder farmers have long used a mixture of plant extracts, including *A. indica*, *T. vogelii*, hot paper, *lantana camara*, and garlic (Julius, 2002). *Tephrosia vogelii* has been utilized as an organic manure in Uganda due to its high phosphorus content (Nakuru, 2013). However, *Spodoptera frugiperda* has not been thoroughly researched in terms of the efficiency of botanical combinations (Julius, 2002). Thus, the purpose of this study is to evaluate the effectiveness of a mixture of *Tephrosia vogelii* and *Azadirachta indica* in controlling *Spodoptera frugiperda* in a lab setting.

Materials and methods

Collection of the plant samples:

T.ogelli and *A. indica* leaves were collected from mature plants in Mpigi and Ibanda district, respectively (00°05.399,032°06.214) and (0.273589 ,30.520363) the coordinates were captured using GPS essentials v.4.4.64. Both plant samples were identified morphologically using the whole plant structures and leaves following standard protocols by (Mkindi et al., 2019; Singh et al., 2017). The leaves were collected before flowering stage, soft branches bearing target leaves were cut using a knife in the morning hours. The samples were then brought to the pharmacy laboratory at Mbarara University, cleaned under running water, sorted to remove foreign materials, and allowed to dry under shade at room temperature for two weeks.

Extraction methods.

Preparation of aqueous extracts

Following the cold infusion method, the dry leaf samples of both *Azadirachta indica* and *Tephrosia vogelii* were ground into powder using an electronic grinder, measured, and packed in airtight containers at room temperature.

100 g of crude leaf powder of each sample were soaked in 500ml of cold distilled water for 12 h at room temperature with constant shaking in the morning and evening after a 12 hours intervals to increase surface area for content extraction (Los, 2010). The mixture was filtered using a muslin cloth to obtain the crude filtrates. The filtrates were then transferred to a freeze dryer at temperature of -82°C for 24hours to stop any reaction. For each of the frozen filtrate, 2g of the crude solid were weighed on an analytical balance and dissolved in 10mls of distilled water to form a test solution for screening.

Preparation of ethanoic extracts

The extraction was done using cold maceration dissolving 250 g of both *Tephrosia vogelii* and *Azadirachta indica* leaf crude powder each in a separate 1000mls of 70% ethanol for 48 hours(Los, 2010). The mixtures were agitated frequently every after 12 hours, to increase surface area for extraction yield. Excess ethanol was evaporated off the solutions using the counter-current method of drying (Yong & IKEGAKI, 1998). From each of the resultant dry solids, 2 g were weighed off using an analytical balance and dissolved in 10 ml of distilled water to make solutions for phytochemical screening.

Phytochemical screening:

Based on the method of extraction, the following tests were applied to examine presence of each of the phytochemicals.

Alkaloids

Following the Dragendorff's, to 2mls of the extract both ethanoic and aqueous in the test-tube was added 1ml of 1% hydrochloric acid followed by three drops of Dragendorff's. In the ethanoic extracts, there were no observable change with *A. indica* and a trace brown precipitate with *T. vogelii* while in the aqueous extract, a trace brown precipitate was observed with *A. indica* and moderate brown precipitation with *T. vogelii*. (Mungole et al., 2010).

Saponins

Following the frothing test, to 2ml of distilled water in test-tube was added 2ml of both the ethanoic and aqueous extracts separately and each shaken vigorously, allowed to stand for 30 minutes. The *A. indica* and *T.vogelii* ethanoic extracts formed a moderate froth and trace froth respectively while in the aqueous a dense froth and moderate froth were formed respectively. (Mungole et al., 2010).

Tannins

Following the ferric test, to 2ml of each of the extracts in the test-tube was added three drops of ferric chloride. The *A. indica* & *T.vogelii* ethanoic and aqueous extracts turned to a blue-black solution. (Mungole et al., 2010)

Terpenoids

Following the Salkowski test, to 2ml of each of the extracts in the test tubes was added 2ml of chloroform and 2ml of concentrated sulphuric acid. The *A. indica* and *T.vogelii* ethanoic extracts formed a moderate red ring and a no red ring formed respectively while in the aqueous a dense red ring and a trace red ring were formed respectively. (Mungole et al., 2010).

Flavonoids

Following the Ammonia test, to each of the aqueous and ethanoic extracts in the test tubes was dipped a filter paper, the filter papers were then exposed to vapors of ammonia solution. The *A. indica* and *T.vogelii* ethanoic extracts formed a pale yellow solution while in the aqueous a dense yellow solution and moderate yellow solution were formed respectively. (Mungole et al., 2010).

Glycosides

Following the Benedict's test, to 2ml of each of the extracts in test tubes, 2ml of Benedict's reagent were added separately and mixtures were heat for 5 minutes, color changes were observed. Both the ethanoic and aqueous extracts of *A. indica* turned to a red solution while the *T.vogelii* extracts turned a green turbid solution. (Mungole et al., 2010).

Laboratory bioassay of botanical extracts against S. frugiperda

Insect colony

A total of 250 third instar *Spodoptera frugiperda* larvae were gathered from an untreated corn field in Uganda's Isingiro district, which is 36 kilometers south of Mbarara (-0.710194,30.734268). Each larva was gathered along with its maize leaf, put in vented paper boxes, and then brought right away to Mbarara University of Science & Technology's biology lab. To prevent cannibalism, each larva was put inside its own ventilated plastic cylinder (diameter). Each larva was given 60g of tender maize leaves that had been macerated and harvested from plants that were 15 to 30 days old(Sisay et al., 2019). Every two days, fresh new leaves were supplied. All the way up to the pre-pupal stage, the larvae were being monitored. For pupation, the pre-pupal larvae were placed to additional plastic cylinders that were a third filled with dirt. The pupae were collected, put in a petri dish that had been moistened, and then placed in an oviposition netted cage with sterile cotton that had been dipped in sugar solution(Sisay et al., 2019). Wax paper along the oviposition cage wall is required for the adults to lay eggs(Sisay et al., 2019). After two to five days, egg batches were removed from the wax paper and put in sterile plastic jars where they were checked each day to see if they were hatching(Sisay et al., 2019). Freshly chopped tender maize leaves were introduced for the larvae to eat as soon as the first instars appeared. Rearing was done at room temperatures and treatments were given to the larvae of the second generation.

2.0 Design The objective of the bioactivity was to assess the efficacy of the combination of *T. vogelli* and *A. indica* leaf extracts in causing S. frugiperda mortality. A complete randomized design was used.

2.1 **Set up and treatment.**

The *S. frugiperda* second generation third instar larvae were placed in plastic cylinders with perforated tops that measured 30 cm x 10 cm x 30 cm for length, diameter, and circumference, respectively. Ten third-instar larvae and 60g of tender, macerated maize leaf, enough to feed the larvae for two days, were included in each cylinder. Each cylinder contained 10 third instar larvae and was randomly allocated one of the following treatments: A, B, C, D, and E. 20g of Tephrosia and 10g of neem leaf powder were included in Treatment A; 10g of Tephrosia and 20g of neem leaf powder were included in Treatment B; 15g of Tephrosia and 15g of neem leaf powder were included in Treatment C; 30g of Tephrosia was included in Treatment D; and 30g of neem leaf powder was included in Treatment E. After being combined with water in a 10:100 (w/v) ratio, the powders were let to stand for 24 hours before being sieved through a muslin fabric. 20ml of the extract was sprayed into each cylinder. When the botanicals were applied separately in treatments D and E, the variations in treatments A, B, and C were designed to understand the effect of combination ratio variations on the percentage mortality of S. frugiperda larvae. Each treatment was replicated three times. Cypermethrin (Rocket) and water was applied in cylinders 6 and 7 as the positive and negative controls, respectively.

Statistical data analysis

In this study, all data was tested for normality using Anderson- Darling test. The variation in the mortality caused by the selected extract treatments were analysed using One-way Analysis of Variance (ANOVA) test in SPSS followed by a POST-HOC Turkey test for the pairwise comparison among the treatment. We employed a Probit regression analysis modal to predict the LT_{50} and LT_{90} of all the selected extract treatments. All the statistical analysis were carried out in Statistical Package for the Social Sciences (SPSS) version 2.0.0 at 95% Confidence level. The results from this study were presented in form of Graphs and tables and summarized using descriptive statistics (mean ± SD).

Results

Bioactive phytochemicals in the aqueous and ethanoic extract of T.vogelii and A.indica leaves. Table 1

Qualitative tests done on *T. vogelii* leaf extracts revealed that the ethanoic extract had abundant of tannins, with moderate flavonoids, trace of alkaloids, saponins, glycosides and terpenoids. The aqueous extract had abundant saponins, tannins, terpenoids, flavonoids and glycosides. (Table 1).

On the other hand, *A. indica* leaf ethanoic extracts had abundant glycoside, moderate saponins, tannins, terpenoids, trace of flavonoids and alkaloids absent. Its aqueous leaf extracts had abundant, tannins, moderate flavonoids and saponins with trace of glycosides and alkaloids.

T. vogelii aqueous extract had significantly higher concentration of flavonoids a chemical group to which a rotenoid deguelin the active pesticidal compound in *T. vogelii* belongs (Zhang et al., 2020) than in its ethanoic extract.

The aqueous extract of *A. indica* as well had a significant concentration of terpenoids higher than in its ethanoic extract, terpenoids is the phytochemical group to which azadiractin the active pesticidal compound in A. indica belongs (Mcgarvey & Croteau, 1995).

The aqueous extract of both the selected botanicals averagely revealed higher concentration of the phytochemicals than the ethanoic extracts. The aqueous extraction in this case the cold infusion was therefore chosen to prepare treatments for the bioactivity tests.

Table 1
Showing Phytochemical results of aqueous and ethanoic extracts of T. vogelii and A. indica

GROUP	**TEST/REAGENT**	**DEDUCTION**			
		NEEM		**TEPHROSIA**	
		Aqueous	**Ethanoic**	**Aqueous**	**Ethanoic**
Alkaloids	Dragendorff's test	+	-	++	+
Saponins	Foam test	++	++	+++	+
Tannins	Ferric Chloride test	+++	++	+++	+++
Terpenoids	Salkowski test	+++	++	+++	+
Flavonoids	Ammonia test	++	+	+++	++
glycosides	Benedicts test	+	+++	+++	+
- represents absent; + represents traces; ++ represents moderate; +++represents abundant. Source: (Mungole et al., 2010)					

Bioactivity of aqueous extract of prepared treatments of *T. vogelii* **and** *A. indica* **against S. frugiperda third instar larvae**

All the treatments were significantly higher than the negative control in causing mortality to *S. frugiperda* at LT_{50} and LT_{90} (F = 15.180; df = 20, p < 0.00). treatment A (41.990 hours) and treatment D (47.785 hours) required averagely less time to cause 50% mortality of the worm compared to treatments B (60.908 hours) and E (63.560 hours) that required much more time to cause 50% mortality to the worm. Treatment C (56.008 hours) required an average time to cause 50% mortality of the worm as compared to all the other treatments.

Table 2
Showing analysis of lethal time of the treatments at 50% and 90% mortality

Treatment	LT_{50} (Hours)	LT_{90} (Hours)
E	63.560	134.961
D	47.785	111.581
B	60.908	131.396
C	56.008	109.073
A	41.990	70.035
Positive control	22.571	35.584
Where: LT means lethal time		

ANOVA

There was significant difference in mortality caused by the treatments to larvae of *S.frugiperda* after 48h ($F = 15.18$; $DF = 20$; $p < 0.00$), (Table 3). Treatments A caused 60% mortality, B (33.33% mortality), C (40% mortality), and D (56.67% mortality), E (23.33%), Treatment A caused the highest percentage mortality at 60% while treatment E caused the lowest percentage mortality at 23.33% after 48h hours of application.

Table 3: Analysis of variance results of the treatments

Treatment	Total Mortality (Mean ± SEM)
A	26.67 ± 1.98
B	0.00 ± 1.98
C	6.67 ± 1.98
D	23.33 ± 1.98
E	33.33 ± 1.98
P	63.33 ± 1.98
ANOVA	
F=	15.18
DF=	20
P=	< 0.00

All the treatments had a reliable relationship of effectiveness in causing mortality to *S. frugiperda* at LT50 and LT90 with treatments A ($R^2 = 0.7576$), E ($R^2 = 0.7147$), B ($R^2 = 0.6991$), D ($R^2 = 0.6428$) and C ($R^2 = 0.56$) Fig. 1 with treatment A showing the most significant efficacy. Treatments with a higher concentration of *T. vogelii* had a higher efficacy in causing mortality to the worms than treatments with more of *A. indica*. This implies that *T. vogelii* has more effectiveness than *A. indica* does.

Discussion

Botanicals have been proven to contain sustainable bioactive phytochemicals against pests of a wide range including worms, arthropods, and mites among others. A number of phytochemical groups including alkaloids, saponins, terpenoids, tannins, flavonoids, glycosides, carbohydrates, phytosterols, caretonoids, anthocyanins, phenols and lignan to mention but a few have been studied to exist in plants, these are found in the different plant parts including roots, the bark and leaves(Belay & Sisay, 2014). The phytochemicals are reported to have pesticidal effects ranging with respiratory blockage, antifeedants, antiovipositionong, interruption of metamorphosis cycles with antimolting properties, hormonal imbalance stimulations leading to physiological disruptions among others (Odeyemi, 2008).

This study focused on two botanicals namely *A. indica* commonly known as neem plant and *T. vogelii* commonly known as fish poison bean, in both, leaves were the main plant part of extraction. The study focused on five phytochemicals namely Alkaloids, Saponins, Terpenoids, Tannins, Flavonoids and Glycosides. In the ethanoic and aqueous extracts of the two plants all the target phytochemicals were present with only the ethanoic extract of the neem plant showing absence of alkaloids. The aqueous extract showed a significant high content of the phytochemicals than the ethanoic extract. In the aqueous extracts, on average *T. vogelii* showed abundant presence of the phytochemical compared to *A. indica* that on average moderately contained the phytochemicals. This explains why cold infusion was selected as a method of preparation of the test treatments

In this study, all the tested treatments were toxic to *S. frugiperda* because they all averagely contained chemical groups of alkaloids, terpenoids, flavonoids, saponins and tannins (Table 1) that have pesticidal effects. Some treatments demonstrated higher mortality levels to the worm than others in the laboratory test. In the laboratory bioactivity tests, a moderate to high percentage mortality of the *S. frugiperda* was observed with treatments D and A respectively. It was noted that the mortality rates of the worm increased with time after application. This possibly indicated a residual effect of the treatments with time. It was also noted that treatments that contained a high portion of *T. vogelii* (A and D) demonstrated a higher efficacy than the other treatments (B and E) with more of neem extracts, It could be so because the aqueous extract of *T. vogelii* showed abundant presence of the bioactive chemicals of terpenoids, tannins, flavonoids and saponins than the *A. indica* extract that contained a moderate of these bioactive phytochemical groups.

The bioactive phytochemicals act diversely in the protection of plants against pests, for example the terpenoids, these are classified in a range of classes namely hemiterpenes, monoterpenes, sesquiterpene, diterpenes, triterpenes and tetraterpenes among others (Ninkuu et al., 2021).

Terpenoids do repelling, anti-feeding and oviposition of herbivores in protection for plants against insect pests. For example, linalool (a monoterpenoid) and (*E*)-β-farnesene (a sesquiterpene) produced by plants repel herbivores and aphids, respectively. Probably the mechanism of action include the inhibition of ATP synthase, alkylation of nucleophiles and interference with molting and hence disrupting the life cycle of the pests. Terpenoids like α-pinene

and β-pinene destabilize the nervous system in insects by inhibition of acetylcholinesterase(Sharma et al., 2017).

The tannins on the other hand which are classified in four groups following their structural characterization that is to say the Gallo- tannins, ellagitannins, complex tannins, and condensed tannins, they bind with proteins affecting the efficacy of nutrient absorption and causing midgut lesions hence negatively impacting the insect growth. they are also bitter polyphenols effecting feeding to many insect pests, moreso when ingested, the tannins minimize the nutritive value of plants to insects through reduction of ability to digest proteins (El-Aswad et al., 2023).

The flavonoids with classes such as flavones, flavonols, flavonones (Elkhidr & Ahmed, 2022). These show pesticidal effects with interference in the of molting and reproduction process of several insects, flavonoids inhibit transcription of the ecdysone hormone at the receptor sites, a juvenile hormone responsible for insect development and stage transition (Nukmal et al., 2017; Zhang et al., 2020).

The saponins classified in two major groups namely the tritepenoid saponins and steroidal saponins(Faizal & Geelen, 2013), they are known by their toxicity to harmful insects causing anti-feeding, disturbance of the moult, growth regulation, mortality among others. This is because they interact with cholesterol, causing a disturbance of the synthesis of ecdysteroids (Geyter et al., 2007).

T. vogelii aqueous extract had significantly high concentration of flavonoids a chemical group to which a rotenoid deguelin the active pesticidal compound in *T. vogelii* belongs (Zhang et al., 2020) than in its ethanoic extract. The aqueous extract of *A. indica* as well had a significant concentration of terpenoids higher than in its ethanoic extract, terpenoids are the phytochemical group to which azadiractin the active pesticidal compound in *A. indica* belongs (Mcgarvey & Croteau, 1995). This explains why cold infusion was selected as a method of treatment preparation as it was predicted to cause a significant bioactive efficacy

Conclusions

The ethanoic and aqueous leaf extracts of both *T. vogelii* and *A. indica* contain pesticidal phytochemicals with the aqueous extract of both *T. vogelii* and *A. indica* containing more phytochemicals. Treatments that constituted a combination of the two plant extracts were more effective in killing *S. frugiperda* than the treatments that constituted a single plant mix. Among the treatments that constituted a combination of *T. vogelii* and *A. indica*, the treatments with a high concentration of *T. vogelii* extracts proved more effective in killing *S. frugiperda* than combined treatments whose highest concentration was of *A. indica.* Pesticidal activities of both *T. vogelii* and *A. indica* are dependent on concentration and time of exposure to the target pests *S. frugiperda* in this case. Further studies are required to isolate and make clear the structure of the pesticidal bioactive molecules in both *T. vogelii and A. indica*, these botanicals also need to be tested for efficacy under field conditions.

Recommendation

The authors recommend extraction of the two selected plant leaves following cold infusion as it was revealed to show more extraction efficacy.

Declarations

Acknowledgments

We extend our sincere appreciation to the Center Of Innovation And Technology Transfer of Mbarara University of Science and Technology for the financial facilitation through their seed grants to starting innovations, the department of pharmacy and biology of the University for offering the team a conducive working space.

Least but by no means last, we appreciate Mr. Ssenkuba Francis and Mr. Nuwagira Upton for tremendous work of guidance and tireless efforts in shaping this work.

Funding

This work was fully funded by the Center for Innovation And Technology Transfer(CITT) of Mbarara University of Science and Technology

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

DATA AVAILABILITY

The data that support the findings of the study are available from corresponding authors N.J and M.R.S upon reasonable request.

References

1. . P. D. P. R. H. V. (2014). Development of Automated Aerial Pesticide Sprayer. *International Journal of Research in Engineering and Technology*, *03*(04), 856–861. https://doi.org/10.15623/ijret.2014.0304151
2. Akinnifesi, F. K., Makumba, W., Sileshi, G. W., & Ajayi, O. C. (2009). Farmer participatory assessment of two researchermanaged 'fertilizer tree systems in Southern Malawi. *African Journal of Agricultural Research*, *4*(4), 269–277.

3. Alao, F. O., & Adebayo, T. A. (2015). Comparative Efficacy of *Tephrosia vogelii* and *Moringa oleifera* against Insect Pests of Watermelon (C*itrullus lanatus* Thumb). *International Letters of Natural Sciences*, *35*, 71–78. https://doi.org/10.18052/www.scipress.com/ilns.35.71
4. Ayilara, M. S., Adeleke, B. S., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, L. A., Omole, R. K., Johnson, R. M., Uthman, Q. O., & Babalola, O. O. (2023). Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Frontiers in Microbiology*, *14*(February), 1–16. https://doi.org/10.3389/fmicb.2023.1040901
5. Belay, K., & Sisay, M. (2014). Phytochemical Constituents and Physicochemical Properties of Medicinal Plant (Moringa Oleifera) Around Bule Hora. *Chemistry and Materials ResearchOnline)*, *6*(7), 2225–2956.
6. Benson, M. F. (2017). Fall armyworm /. *Fall Armyworm /*, 6. https://doi.org/10.5962/bhl.title.123807
7. Bhusal, S., & Chapagain, E. (2020). Threats of fall armyworm (Spodoptera frugiperda) incidence in Nepal and it’s integrated management-A review. *Journal of Agriculture and Natural Resources*, *3*(1), 345–359. https://doi.org/10.3126/janr.v3i1.27186
8. Carvalho, F. P. (2017). *Pesticides , environment , and food safety*. https://doi.org/10.1002/fes3.108
9. Dad, K., Zhao, F., Hassan, R., Javed, K., Nawaz, H., Saleem, M. U., Fatima, T., & Nawaz, M. (2022). Pesticides Uses, Impacts on Environment and their Possible Remediation Strategies-A Review. *Pakistan Journal of Agricultural Research*, *35*(2), 274–284. https://doi.org/10.17582/JOURNAL.PJAR/2022/35.2.274.284
10. Divekar, P. (2023). *Botanical Pesticides : An Eco-Friendly Approach for Management of Insect Pests*. *7*(2), 75–81.
11. El-Aswad, A. F., Aisu, J., & Khalifa, M. H. (2023). Biological activity of tannins extracts from processed Camellia sinensis (black and green tea), Vicia faba and Urtica dioica and Allium cepa essential oil on three economic insects. *Journal of Plant Diseases and Protection*, *130*(3), 495–508. https://doi.org/10.1007/s41348-022-00680-x
12. Elkhidr, H. E., & Ahmed, T. M. (2022). Classification and Identification of Flavonoids from plant kingdom as Acacia species. *International Research Journal of Engineering and Technology*, *May*, 3353–3361. www.irjet.net
13. Faizal, A., & Geelen, D. (2013). Saponins and their role in biological processes in plants. *Phytochemistry Reviews*, *12*(4), 877–893. https://doi.org/10.1007/s11101-013-9322-4
14. Gadzirayi, C. T., Mutandwa, E., Mwale, M., & Chindundu, T. (2009). Utilization of Tephrosia vogelii in controlling ticks in dairy cows by small-scale commercial farmers in Zimbabwe. *African Journal of Biotechnology*, *8*(17), 4134–4136.
15. Geyter, E. De, Lambert, E., Geelen, D., & Smagghe, G. (2007). *Novel Advances with Plant Saponins as Natural Insecticides to Control Pest Insects*.
16. Gobernment of Wetern Australia. (2020). Fall armyworm larval identification guide. *Department of Primary Industries and Regional Develpment*, *August*, 1–3.

17. Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of the fall armyworm spodoptera frugiperda (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE*, *11*(10), 1–9. https://doi.org/10.1371/journal.pone.0165632
18. Grzywacz, D., Stevenson, P. C., Mushobozi, W. L., Belmain, S., & Wilson, K. (2014). The use of indigenous ecological resources for pest control in Africa. *Food Security*, *6*(1), 71–86. https://doi.org/10.1007/s12571-013-0313-5
19. Jallow, M. F. A., Awadh, D. G., Albaho, M. S., Devi, V. Y., & Thomas, B. M. (2017). *Pesticide Knowledge and Safety Practices among Farm Workers in Kuwait : Results of a Survey*. https://doi.org/10.3390/ijerph14040340
20. Jeyaratnam, J. (1990). Acute pesticide poisoning - A global public health problem. *Danish Medical Bulletin*, *54*(1), 58–59.
21. Journal, T., & June, T. (2013). *T-Institute Table Of Contents : 3*(6).
22. Julius, M. (2002). *Pesticidal Plants Used in Masaka District of Uganda*. *2*(1).
23. Kamanula, J., Sileshi, G. W., Belmain, S. R., Sola, P., Mvumi, B. M., Nyirenda, G. K. C., Nyirenda, S. P., & Stevenson, P. C. (2011). Farmers' insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Africa. *International Journal of Pest Management*, *57*(1), 41–49. https://doi.org/10.1080/09670874.2010.522264
24. Kamatenesi, M. M., Deng, A. L., J.O.Ogendo, E.O.Omolo, M.J.Mihale, M.Otim, Buyungo, J. P., & Bett, P. K. (2008). Indigenous knowledge of field insect pests and their management around lake Victoria basin in Uganda. *African Journal of Environmental Science and Technology*, *2*(8), 342–348. http://www.academicjournals.org/AJest
25. Kenko, D. B. N., & Kamta, P. N. (2021). Human and Environmental Health Implications of Pesticide Utilization by Market Gardeners in the Western Highlands of Cameroon. *Asian Journal of Environment & Ecology*, *14*(3), 44–56. https://doi.org/10.9734/ajee/2021/v14i330210
26. Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Mendesil, E., Gohole, L., & Tefera, T. (2019). Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (Spodoptera frugiperda) in Ethiopia and Kenya. *International Journal of Pest Management*, *65*(1), 1–9. https://doi.org/10.1080/09670874.2017.1423129
27. Los, U. M. D. E. C. D. E. (2010). *No Title*.
28. Luntz, A. J. M., & Nisbet, A. J. (2000). *Azadirachtin from the Neem Tree Azadirachta indica : its Action Against Insects*. *29*(4), 615–632.
29. Mcgarvey, D. J., & Croteau, R. (1995). *Terpenoid Metabolism*. *7*(July), 1015–1026.
30. Mkindi, A. G., Tembo, Y., Mbega, E. R., Medvecky, B., Kendal-smith, A., Farrell, I. W., Ndakidemi, P. A., Belmain, S. R., & Stevenson, P. C. (2019). Phytochemical analysis of tephrosia vogelii across east africa reveals three chemotypes that influence its use as a pesticidal plant. *Plants*, *8*(12), 1–11. https://doi.org/10.3390/plants8120597

31. Mungole, A. J., Awati, R., Chaturvedi, A., & Zanwar, P. (2010). Preliminary phytochemical screening of Ipomoea obscura (L) -a hepatoprotective medicinal plant. *International Journal of PharmTech Research*, *2*(4), 2307–2312.
32. Nakuru, K. (2013). *Joint Proceedings of the 27th Soil Science Society of East Africa and the 6th African Soil Science Society Conference Theme : " Transforming rural livelihoods in Africa : How can land and water management contribute to. October*. http://library.wur.nl/isric/fulltext/isricu_i34330_001.pdf
33. Navasero, M. M., & Navasero, M. V. (2020). Life cycle, morphometry and natural enemies of fall armyworm, spodoptera frugiperda (J.E. Smith) (lepidoptera: Noctuidae) on zea mays l. in the Philippines. *Journal of the International Society for Southeast Asian Agricultural Sciences*, *26*(2), 17–29.
34. Ninkuu, V., Zhang, L., Yan, J., Fu, Z., Yang, T., & Zeng, H. (2021). *Biochemistry of Terpenes and Recent Advances in Plant Protection*.
35. Nukmal, N., Rosa, E., Apriliyani, & Kanedi, M. (2017). Insecticidal effects of the flavonoid-rich fraction of leaves extract of gamal (Gliricidia sepium) on the coffee mealybugs (Planococcus citri risso.). *Annual Research and Review in Biology*, *16*(6), 1–9. https://doi.org/10.9734/ARRB/2017/36209
36. Odeyemi, O. (2008). A review of the use of phytochemicals for insect pest control. *African Plant Protection*, *14*, 1–7. http://reference.sabinet.co.za/sa_epublication_article/plantpro_v14_a1
37. Pandey, P., Chakole, S., Wanjari, M. B., & Prasad, R. (2023). *A Bibliometric Analysis of Scientific Research Publications Related to Pesticide Poisoning in the South Asian Countries*. *15*(4), 8–12. https://doi.org/10.7759/cureus.38134
38. Raven, P. H., & Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(2), 1–6. https://doi.org/10.1073/PNAS.2002548117
39. Rojas, J. C., Virgen, A., & Malo, E. A. (2004). Seasonal and nocturnal flight activity of Spodoptera frugiperda males (Lepidoptera: Noctuidae) monitored by pheromone traps in the coast of Chiapas, Mexico. *Florida Entomologist*, *87*(4), 496–503. https://doi.org/10.1653/0015-4040(2004)087[0496:SANFAO]2.0.CO;2
40. Sagar, G. C., Aastha, B., & Laxman, K. (2020). An introduction of fall armyworm (Spodoptera frugiperda) with management strategies: a review paper. *Nippon Journal of Environmental Science*, *1*(4), 1010. https://doi.org/10.46266/njes.1010
41. Samada, L. H., & Tambunan, U. S. F. (2020). Biopesticides as promising alternatives to chemical pesticides: A review of their current and future status. *OnLine Journal of Biological Sciences*, *20*(2), 66–76. https://doi.org/10.3844/ojbsci.2020.66.76
42. Sharma, E., Anand, G., & Kapoor, R. (2017). Terpenoids in plant and arbuscular mycorrhiza-reinforced defence against herbivorous insects. *Annals of Botany*, *119*(5), 791–801. https://doi.org/10.1093/aob/mcw263

43. Singh, A. K., Sharma, R. K., Sharma, V., Singh, T., Kumar, R., & Kumari, D. (2017). Isolation, morphological identification and in vitro antibacterial activity of endophytic bacteria isolated from Azadirachta indica (neem) leaves. *Veterinary World*, *10*(5), 510–516. https://doi.org/10.14202/vetworld.2017.510-516
44. Sisay, B., Tefera, T., Wakgari, M., Ayalew, G., & Mendesil, E. (2019). The efficacy of selected synthetic insecticides and botanicals against fall armyworm, spodoptera frugiperda, in maize. *Insects*, *10*(2). https://doi.org/10.3390/insects10020045
45. Sustainable, I. T., & Goals, D. (2021). *Country Situation Report on Highly Hazardous Pesticides (HHPs) in Uganda*. *May*.
46. Volp, T. M., & Myron, P. (2022). *Special Collection : World-Scale Ecology and Management of Fall What Defines a Host ? Oviposition Behavior and Larval Performance of Spodoptera frugiperda (Lepidoptera : Noctuidae) on Five Putative Host Plants*. *115*(6), 1744–1751.
47. Wahaab, R. A., & Badawy, M. I. (2004). Water quality assessment of the River Nile system: An overview. *Biomedical and Environmental Sciences*, *17*(1), 87–100.
48. Yigezu, G., & Wakgari, M. (2020). *Local and indigenous knowledge of farmers management practice against fall Local and indigenous knowledge of farmers management practice against fall armyworm (Spodoptera frugiperda) (J . E . Smith) (Lepidoptera : Noctuidae): A review*. *January*.
49. Yong, K. P., & IKEGAKI, M. (1998). .Pdf. In *Bioscience, biotechnology, and biochemistry* (Vol. 62, Issue 11, pp. 2230–2232).
50. Zhang, P., Qin, D., Chen, J., & Zhang, Z. (2020). Plants in the genus tephrosia: Valuable resources for botanical insecticides. *Insects*, *11*(10), 1–18. https://doi.org/10.3390/insects11100721

Figures

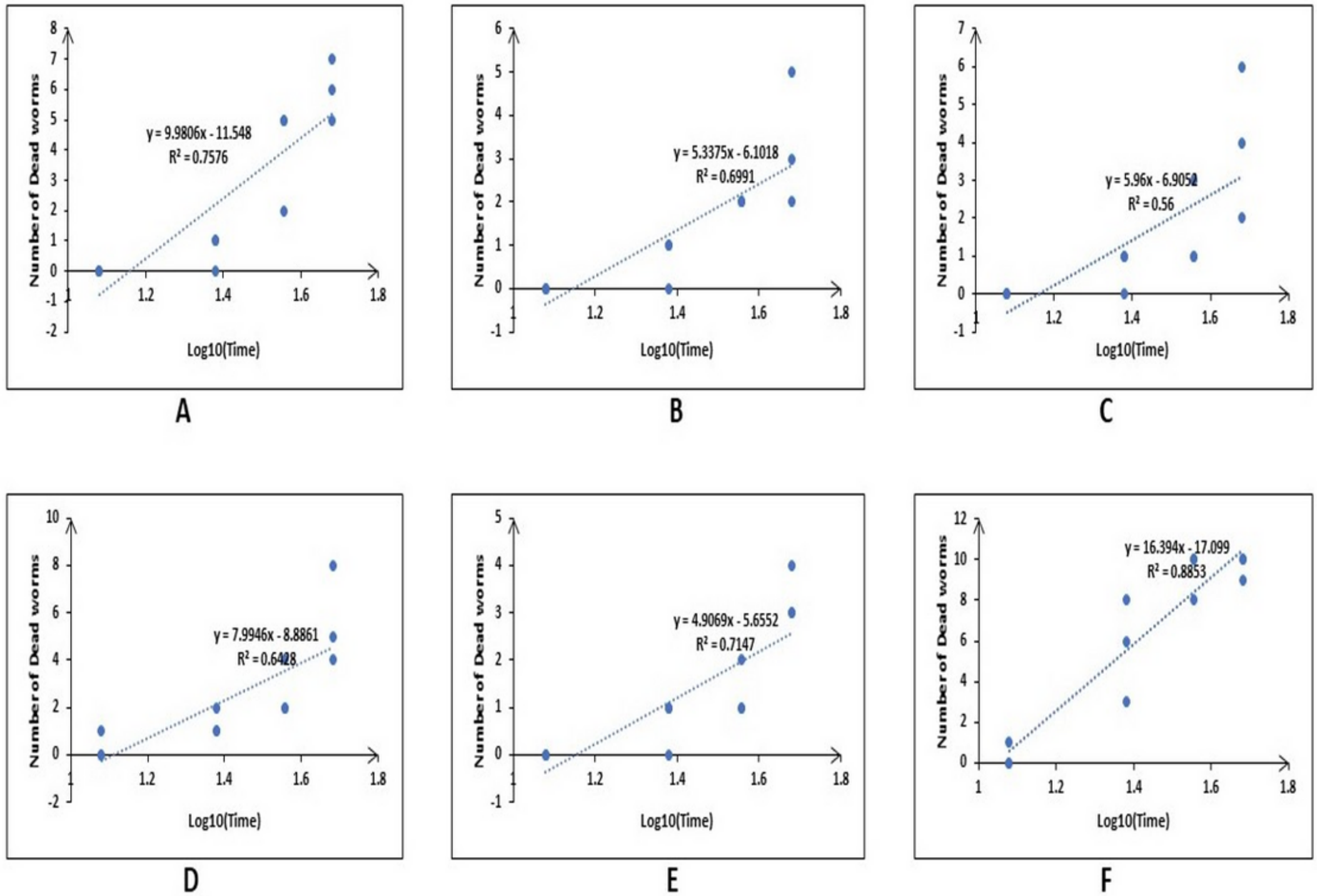

Figure 1

Showing Probit regression modal equations used to predict LT50 and LT90 for: A- Tephrosia 20g and Neem 10g, B- Neem 20g and Tephrosia 10g, C-Neem 15g and Tephrosia 15g, D- Tephrosia_30g, E- Neem_30g and F- Positive control.