Agricultural Science; Vol. 2, No. 1; 2020
ISSN 2690-5396 E-ISSN 2690-4799
https://doi.org/10.30560/as.v2n1p47

Biopesticidal Properties of Aqueous Crude Extracts of Tobacco (*Nicotiana Tabacum* L.) Against Fall Armyworm (*Spodoptera Frugiperda* J.E Smith) on Maize Foliage (*Zea Mays* L.) Diets

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Received: January 28, 2020 Accepted: February 28, 2020 Online Published: March 29, 2020

Abstract

Pesticidal plants offer valuable and sustainable options for managing Lepidopteran pests with considerable health, environmental and economic benefits in smallholder agro-ecosystem. Research was done to determine the efficacy of aqueous extracts of tobacco (Nicotiana tabacum) against fall armyworm (Spodoptera frugiperda Smith) on maize foliage (Zea mays L.) diets. Bio-efficacy of aqueous crude *N. tabacum* leaf extracts was evaluated under average room temperature at Great Zimbabwe University, Biology laboratory. The treatments were tobacco leaf extracts at four dosage levels (25 %, 33.33%, 41.67 % and 50% W/V) and a negative control of untreated maize leaf foliage (distilled water) was used. A positive control of Carbaryl 85% WP was also used at label rates. The experiment was arranged in a Complete Randomized Design (CRD) replicated three times. Five larvae were placed into each of the experimental jars with maize foliage diets drenched into 10ml of distilled water in each treatment extract to keep the maize leaf foliage moist. Mortality for each treatment was recorded at 2 hourly intervals for 20 hours. Results showed that tobacco crude aqueous leaf extracts had antifeedent activities against FAW larvae. The highest dose of 50% had significantly higher mean FAW larval mortalities ($p< 0.05$) than lower dosage (25%) and the negative control after 20 hours. However, 50% concentration was not significantly different ($p>0.05$) from the positive control and that of 33.33% and 41.67% dosages. The bioassay indicated that the 33.33% extract was superior in toxicity to 25% dose and the negative control but similar to higher extract doses though inferior to positive control. However, the mean mortality of 50% extract was not significantly different from that of the positive control. This study recommends that 50% tobacco aqueous crude leaf extract dose to be used when controlling FAW in maize in the smallholder sector.

Keywords: S. frugiperda, Efficacy, Bio efficacy, N. tabacum, Smallholder sector

1. Introduction

Maize (*Zea mays* L.) forms an essential component of the global food security as a major part of the diet of millions of people including Zimbabwe (CIMMYT, 2017). It is also an important livestock feed both as silage and as crop residue or grain and is also used industrially for starch and oil extraction.

In the 2016/2017 season, maize production went down to 35%, which was one third lower than the previous season (FAO, 2017). Currently, average maize yield is at 0.8t/ha which is far below the expected average of 4t/ha (CIMMYT, 2017). The decline is attributed to late planting due to unavailability of seed on the market on time, unaffordable prices of inputs e.g. fertilizers for most small holder farmers, shortage of draft power, erratic rainfall and pest infestations. According to FAO STAT (2009), Zimbabwe needs 1.7 million tonnes of maize for direct human consumption per year and according to WHO, maize consumption per day per person is 241g. There is therefore great need to guard against losses that may befall the maize crop in the field. Between 20-40% maize losses are caused by field pest in the tropics (Moris, 2001). This is a cause for concern on guarding against field losses mainly caused by insect pests. Major field pests threatening food security include the maize stalk borer, African armyworm and the fall armyworm (*Spodoptera frugiperda*). In Zimbabwe, the maize crop has suffered severe attack in the past three consecutive agricultural seasons from the problem pest, *S. frugiperda* (Prasanna *et al*, 2018).

According to Eric and John (2017: 57), *S. frugiperda* is a rampant cereal field pest that has resulted in between 40-90% losses in yield. Zimbabwe small scale and commercial farmers currently rely on synthetic chemicals in controlling this pest. Most Zimbabwean farmers are also poor resource farmers who cannot afford buying the chemicals which are expensive and not readily available in most rural markets, besides they are a health hazard to applicators, consumers and the environment (Prakash and Rao, 2007). The destruction of non-target species as well as pesticide resistance is cause for concern in using these synthetic chemicals. Therefore it is very imperative to research on locally available botanicals to replace or supplement the chemical control method. The research seeks to investigate the Antifeedant properties of N. *tabacum* in the control of fall army worm.

Some botanical control methods researched in maize field pests include the use of neem (*Azadirachta indica*), Acacia (*Acacia spp*), Fish-poison bean (*Tephrosia vogelli*) and tobacco (*Nicotiana tabacum*) leaf extracts (Isman, 2010).

2. Materials and Methods

2.1 Experimental Site

The experiment was carried out at the Great Zimbabwe University, Biology laboratory. The University is located 10 kilometres South East of Masvingo town and in agro-ecological region IV of Zimbabwe. The Biology laboratory is located at 20º6.137'S and longitude 30º51.688'E. Soil texture is sandy clay loam to sandy loam (alluvial) with clay content of 34% (AGRITEX department, 2017). Rainfall is erratic, characterized by frequent dry spells. The area receives an average of 450-650mm rainfall per annum. Mean annual temperature ranges from 15^{O}C to 25^{O}C (Meteorological Service Department, 2017).

Figure 1. Location site of Great Zimbabwe University. Source: Google Maps

2.2 Experimental Design

The experiment was arranged in a Complete Randomized Design (CRD) with six treatments replicated three times as illustrated by Gomez and Gomez (1984). The experimental treatments were arranged as shown below:

Table 1. Treatment description

	treatment description
T1	75g N. tabacum leaf extracts mixed to 300ml distilled water to give a 25% concentration (W/V).

T2	100g *N.tabacum* leaf extracts mixed to 300ml distilled water to give a 33.33% concentration (W/V).
T3	125g *N. tabacum* leaf extracts mixed to 300ml distilled water to give a 41.67% concentration (W/V).
T4	150g *N. tabacum* leaf extracts mixed to 300ml distilled water to give a 50% concentration (W/V).
T5	Carbaryl 85% WP mixed at label instructions (150g/15 liters of water).
T6	No *N. tabacum* leaf extracts (control)

The experiment followed a protocol by Birhanu *et al*., (2019:16), who postulated that tobacco leaf powder can be mixed at a rate of 75g crude tobacco leaves to 300ml distilled water to obtain an effective concentration for the control of fall armyworm.

2.3 Experimental Procedure

2.3.1 Collection and Preparation of Crude Tobacco Leaf Extracts

Scrap flue cured top Virginia tobacco leaves, collected from a farm in Gororo area, ward 26 of Chivi district, were crushed into a powder using the Kasa Maria pestle and mortar. The leaf powder was weighed using a digital scale to get the required quantities for the four treatments as shown on Figure 3.2(a). The powder of tobacco leaf was socked in 300ml distilled water for 24 hours at the effective rate previously reported by different authors for Lepdopteran larvae as described above (Table 1) (Birhanu, 2018). The different extracts were then filtered through a cheese cloth to remove tobacco leaf sediments into 1000cm^3 litre beakers and the solutions were left overnight (Birhanu *et al*., 2019). Carbaryl 85% WP was also mixed with water according to the recommended dosage rates (150g/15 litres of water).

2.3.2 Collection and Preparation of Maize Leaf Foliage

Untreated maize leaf foliages, collected from the University campus fields, were cut into pieces of 6cm by 3cm to get 5,6g per treatment following FAW rearing experience that shows that 60g of maize leaf foliage can feed approximately 15 larvae for 2 to 3 days (Birhanu *et al*., 2019). The leaf foliages were then soaked in various treatment solutions (25 %, 33.33%, 41.67 % and 50% W/V) for thirty minutes before being placed into clean 500cm^3 beakers to determine Antifeedent activity of fall armyworm larvae.

2.3.3 Collection of FAW Larvae

Third instar larvae of *S. frugiperda* were collected from Nyamakwe irrigation scheme in Chivi district on the day of the experiment and continuously fed with untreated maize foliage before they were introduced to various experimental treatments.

2.3.4 Prepared Tobacco Concentrations

A total of 18 beakers were used since each treatment was replicated three times and each beaker with 10 maize leaf portions was placed. More leaf portions were continuously added to make sure that feed was always available for the pest. The beakers were arranged in a Complete Randomized Design (CRD) and five 3rd instar larvae were immediately introduced to each beaker with feed. The various treatments were then observed and recorded at two hour intervals for 20 hours.

2.4 Data Collection

2.4.1 Fall Army Worm Mortality

Number of dead caterpillars from each beaker was recorded cumulatively after every 2 hours. Confirmation of death of the caterpillars was done by pricking the insect body with a needle or a larva was considered dead if it could not right itself after being placed on its dorsal surface. FAW percentage mortality for each treatment was assessed as: (number of dead FAW larvae/total number of pests) x100.

2.5 Data Analysis

Collected data was transformed using the square root transformation. The significance level was set at 5%, and the means were separated using the Fisher's Protected Least Significant Differences (LSD) (Gomez and Gomez, 1984). All statistical analyses were performed using the GenStat Release 14.1 statistical software package.

3. Results

3.1 Effects Of N. Tabacum Extracts on S. Frugiperda Mortality in Maize

Mortality results showed that crude aqueous leaf extracts of tobacco were insecticidal against *S. frugiperda.* There were significant differences ($p<0.05$) amongst the treatments as shown on Table 2.

Table 2. Table of *Spodoptera frugiperda* mortality.

	Mortality			
Treatment	**2**	**8**	**14**	**20**
25 % concentration	0.00a	0.00a	0.67ab	1.67ab
33.3 % concentration	0.00a	0.33ab	1.67ab	2.33abc
41.67 % concentration	0.00a	1.00ab	1.67ab	2.33abc
50 % concentration	0.00a	1.33bc	2.67b	2.33abc
Carbaryl 85 % WP	0.33a	2.33c	3.00b	4.67bc
Negative control	0.00a	0.00a	0.00a	0.00a
Grand mean	0.06	0.83	1.61	2.55
pvalue	0.5	0.0003	0.016	0.03
LSD	0.84	1.03	1.68	2.14
CV %	25	14	12	9

*Means followed by the same letter are not significantly different from each other at 5% significance level.

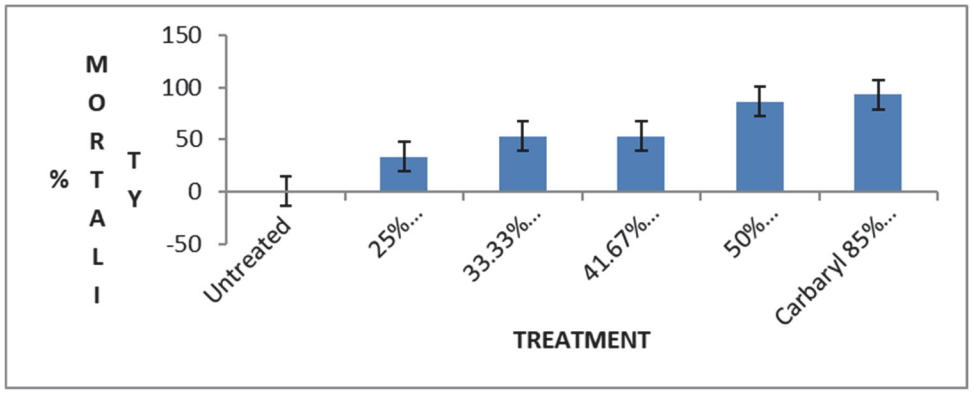

Figure 2. Figure showing *S. frugiperda* mortality on maize foliage

3.2 Effects of Exposure Time on S. Frugiperda Mortality

Time taken by different tobacco concentrations to cause FAW mortality was significantly different ($p<0.05$). After 20 hours of the experiment, cumulative deaths were significantly different ($p<0.05$) from those in the first two hours. However, four hours after the application of the botanical insecticide, the highest tobacco concentration of 50% showed some positive effects as an average total of two caterpillars died.

3.3 Cumulative Mortalities of FAW after Exposure to N. tabacum Concentrations at 8 hours

Results from the experiment for tobacco leaf extract concentrations eight hours after establishment of the trial indicated that there was a significantly higher mortality difference ($p<0.05$) between the lowest concentration of 25% and the highest concentration of 50%. However, there were no significant differences ($p>0.05$) amongst the 50% concentration, 33.33% and 41.67% concentrations.

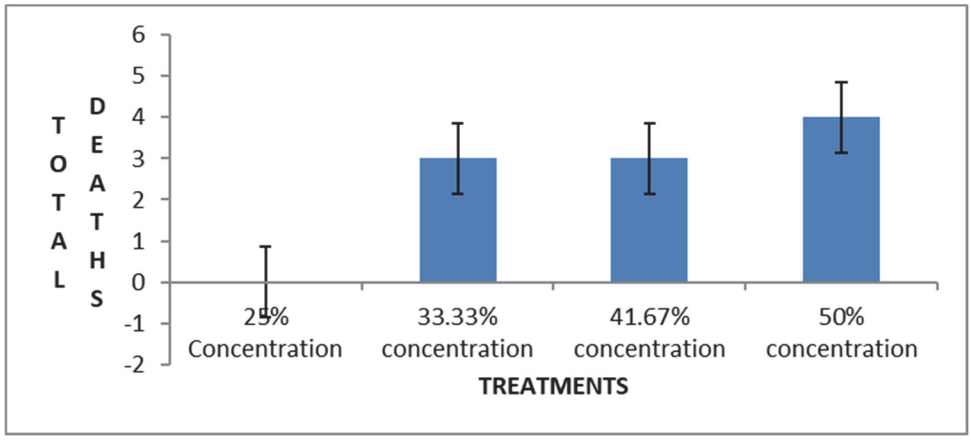

Figure 3. Cumulative mortalities of FAW after exposure to *N. tabacum* concentrations at 8 hours

3.4 Cumulative Mortalities of FAW After Exposure to N. Tabacum Concentrations at 10 Hours.

Results for tobacco leaf extract concentrations ten hours after the start of the trial showed that there was a significantly higher mortality difference ($p<0.05$) between the lowest concentration of 25% and highest concentration of 50%. However, there were no significant differences ($p>0.05$) amongst the 50%, 33.33% and the 41.67% concentrations.

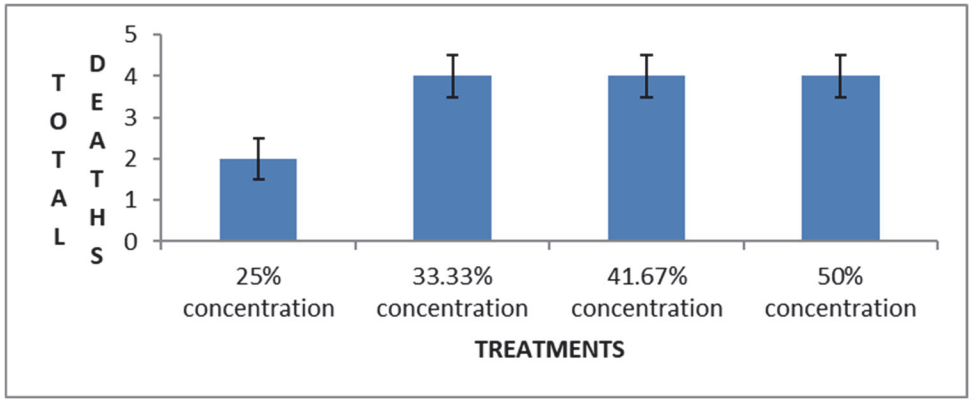

Figure 4. Cumulative mortalities of FAW after exposure to *N. tabacum* concentrations at 10 hours

3.5 Cumulative Mortalities of FAW After Exposure to N. Tabacum Concentration at 12 Hours.

Results from the experiment twelve hours after the start of the trial indicated that there was a significantly higher mortality difference ($p<0.05$) between the lowest concentration of 25% and all the other three treatments. However, there were no significant differences ($p>0.05$) amongst the 50%, 33.33% and the 41.67% treatments.

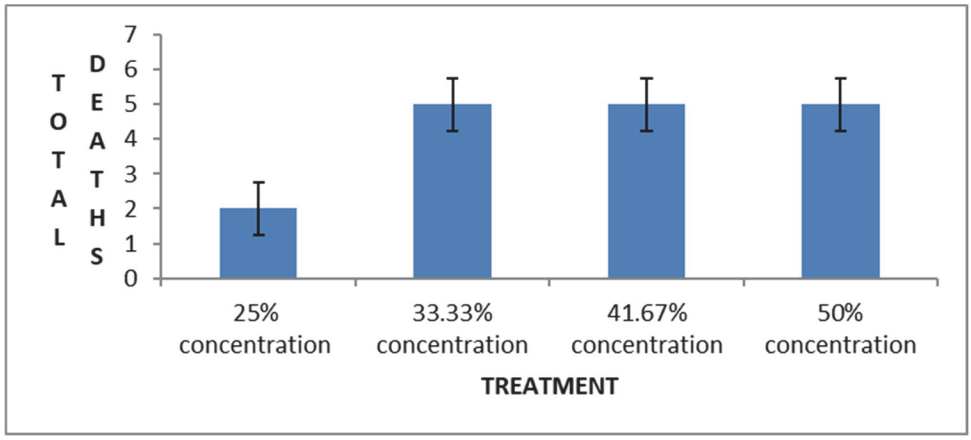

Figure 5. Cumulative mortalities of FAW after exposure to N. tabacum concentrations at 12 hours

3.6 Cumulative Mortalities of FAW After Exposure to N. Tabacum Concentrations at 14 Hours

Results from the experiment for tobacco leaf extract concentrations fourteen hours after the beginning of the trial showed that there was significantly higher mortality difference ($p< 0.05$) between the lowest concentration of 25% and the 50% concentration. However, there were no significant differences ($p>0.05$) amongst the 33.33%, 41.67% and 50% concentrations.

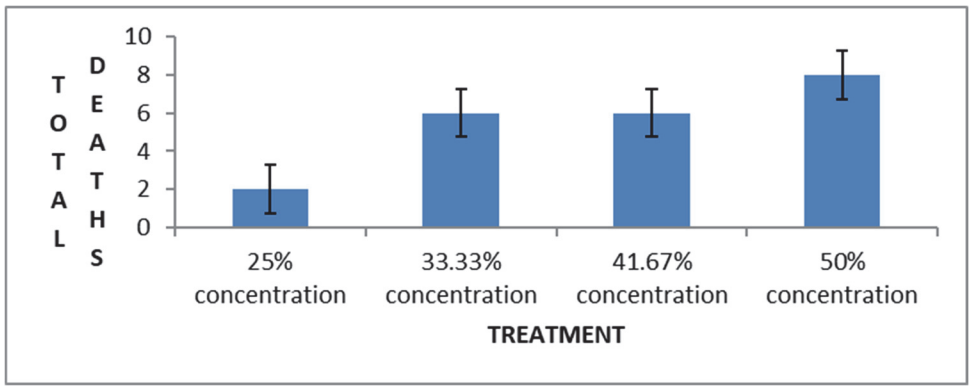

Figure 6. Cumulative mortalities of FAW after exposure to N. tabacum concentrations at 14 hours

3.7 Cumulative Mortalities of FAW After Exposure to N. Tabacum Concentrations at 16 Hours

Results sixteen hours after the beginning of the trial indicated that there was significantly higher mortality difference ($p<0.05$) between the 25% concentration and the highest concentration of 50%. However, there was a significantly lower mortality difference ($p<0.05$) between the 33.33% and the 50% concentration.

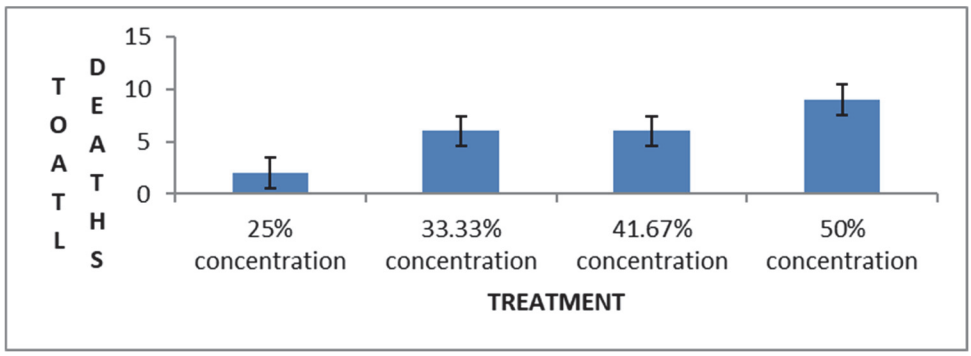

Figure 7. Cumulative mortalities of FAW after exposure to *N. tabacum* concentrations at 16 hours

3.8 Cumulative Mortalities of FAW After Exposure to N. Tabacum Concentrations at 18 Hours

Results from the experiment for tobacco leaf extract concentrations eighteen hours after the start of the trial showed that there was significantly higher mortality difference ($p<0.05$) between the 25%, 33.33%, 41.67% concentrations and the highest concentration of 50%. However, there were no significant differences ($p>0.05$) between the 25% and the 41.67% concentration.

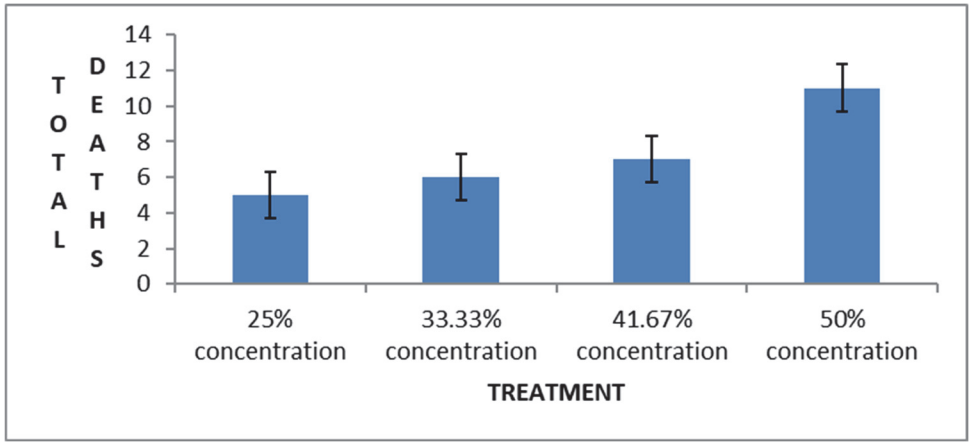

Figure 8. Cumulative mortalities of FAW after exposure to *N. tabacum* concentrations at 18 hours

3.9 Cumulative Mortalities of FAW after Exposure to N. Tabacum Concentrations at 20 hours

There was a significantly higher mortality difference ($p<0.05$) between the highest tobacco leaf extract concentration of 50% and all the other three treatments of 25%, 33.33% and 41.67% respectively.

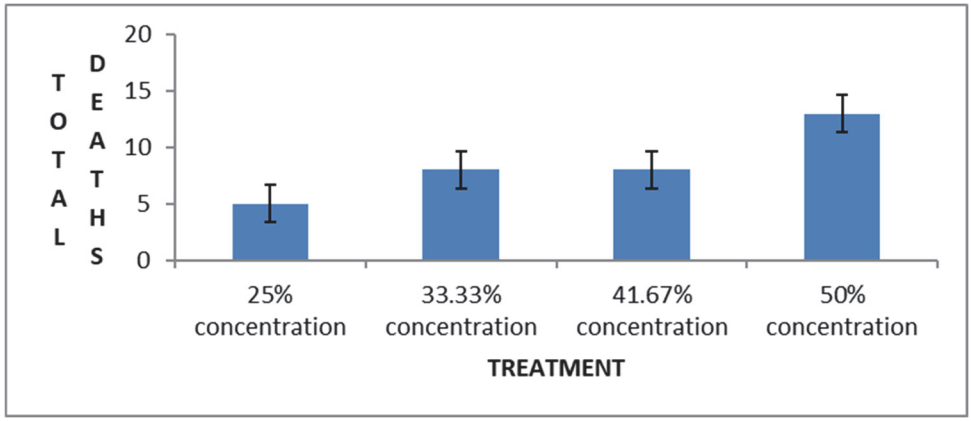

Figure 9. Cumulative mortalities of FAW after exposure to *N. tabacum* concentrations at 20 hours

4. Discussion

4.1 Effects of N. Tabacum Concentration on Mortality of Fall Armyworm

Results from the study indicated that crude aqueous *N. tabacum* leaf extracts can be used effectively to control *S. frugiperda* on maize diets. The results showed that mortality was dose dependent as more caterpillar deaths were recorded at higher dosage levels. This agrees with observations by Silva *et al*., (2019), who revealed that *N. tabacum* leaves contain high nicotine which is toxic to insects. Nicotine exhibits a systemic form of pesticidal effects as supported by (Jarrod *et al.,* 2019). Neonicotinoids, like nicotine, bind to nicotic acetyleholine receptors (nAChRs) of a cell and trigger a response by that cell. In insects, (like FAW), these receptors are found in the central nervous system. This effect of tobacco on insects was also described by Mordue and Nisbert (2010) as a deterrent to feeding, interfering mainly in the physiology of the ecdysis and in cellular processes, potentially resulting in the death of the insect. Low to moderate activation of cell receptors by nicotine causes nervous stimulation while high levels over-stimulate and block the receptors causing paralysis and death of the insect.

4.2 Effects of Exposure Time of Aqueous Tobacco on FAW Mortality

In the current study, the time taken by different tobacco concentrations to kill the pest was significantly different ($p<0.05$). Results indicated that longer exposure periods of FAW to tobacco extracts increases the number of pest mortalities. This was supported by Cresswell *et al*., (2012), who highlighted that the longer the pest is subjected to an aqueous extract from tobacco leaves the higher the number of FAW mortalities as the effect is on the time nicotine takes to terminate the signals from cell receptors. Similar experiments by Birhanu *et al*., (2019) also showed that tobacco leaf extracts can effectively control *S. frugiperda* 72 hours after the pest is exposed or introduced to the botanical insecticide on maize diets.

The effect of time on the control of FAW can be attributed to the stage of controlling the pest. As the pest grows older, a longer time period may be taken to control it due to its resistant characteristics to insecticides as a result of its continued molting or ecdysis. In the experiment, 3rd instar larvae were used. However, Silva *et al*., (2019) highlighted that newly hatched caterpillars, 1st to 2nd instars, can be killed quickly using tobacco.

Results also showed that tobacco extracts require a pre-determined time period to show its efficacy on FAW. However, the longer the time any insecticide takes to completely control the pest, the more damage the pest causes to the maize crop as it feeds continuously (Birhanu *et al*., 2019).

5. Conclusion

Results from the study indicated that tobacco leaf extracts are effective in controlling FAW especially at 50% concentration. Results also show that FAW exposure time to the botanical pesticide is important as cumulative deaths were increasing with more exposure time. After 20 hours, there were more deaths from all tobacco concentrations than at 2 hours after the onset of the experiment.

6. Recommendations

The study recommends that smallholder farmers should use the most effective tobacco concentration of 50% to control fall armyworm. Further studies of using higher tobacco concentrations should also be done and this will not have any cost implication to the smallholder farmer since scrap flue cured tobacco leaves are readily available. Experiments based on more exposure time of the pest to tobacco concentrations should also be done so as to attain 100% pest mortalities. Whilst this was a laboratory experiment, the study also recommends that it be done as a field experiment so that there will be a validation of the pest leaf damage per given time. The study further recommends the use of bioassays with organic solvents as opposed to water extracts.

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