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In vitro determination of LD_{50} for AgriX365 on corn strain of *Spodoptera frugiperda*

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Abstract
Fall armyworm, *Spodoptera frugiperda,* is currently a major and invasive pest that is threatening global maize production and food security especially in sub–Saharan Africa. Its tolerance to several available synthetic pesticides has led to research into novel pest management strategies. An *in vitro* laboratory bioassay was conducted at Bindura University of Science Education to determine the LD_{50} for AgriX365, a botanical pesticide, in a complete randomised design.
There was a relative dose-dependent response in mortality rates of *S. frugiperda* from 0.5ml/L to 2ml/L of AgriX365. The logistic function Probit, *P*, calculation indicated an LD_{50} =1.25ml/L (1250 ppm); $y=0.5998x+4.2749$; R2=0.9979) of the standard or recommended field rate for AgriX365. There was a high significant difference between mean per cent mortality between treated *S. frugiperda* larvae and the untreated ones ($p<0.001$; df=3; F=36.91) and between AgriX365 treatments ($p<0.01$).
Therefore, the standard application rate for AgriX365 is 1.25ml/L, for 50% *of S. frugiperda* larvae to die.

Keywords: Bioassay, botanical pesticide, mortality, fall armyworm

Introduction

Maize is one of the major staple crops grown in Africa, south of the Sahara. However, its production is threatened by the fall armyworm, *Spodopotera frugiperda* (J.E. Smith) (Lepidoptera: Noctuiidae). Respective departments and research institutions in various countries give a full description of the *S. frugiperda*, for example, Department of primary industries and regional development in Australia (2020), Oklahoma State University, USA (2022). *S. frugiperda*, is a deadly emerging and migratory invasive pest that has since proved to be a major problem in maize production, impacting negatively on food security and family income.

Native to the tropical regions of the Western Hemisphere (Nagoshi *et al*., 2021) [12], the pest was noted for the first time in West Africa in early 2016 (Georgen *et al*., 2016) [8]. Since then, its incidence has been recorded in all the countries in Sub-Saharan Africa, except for Lesotho. In Zimbabwe it was detected for the first time in the 2016/2017 season (FAO, 2020) [6]. The pest was detected in Asia from 2016 and by January 2019 it had spread to Bangladesh, China, Myanmar, Thailand, and Sri Lanka (Nagoshi *et al*. 2020; Prasanna *et al*., 2021). Pest forecasts and modelling studies have shown significant likelihood of near global invasion of fall armyworm (Early *et al*., 2018) [5]. Due to the significant crop damage caused by this pest, some countries resorted to the introduction or enhanced use of synthetic chemical pesticides, a development which has placed the economic viability of small-scale cropping systems at risk. The resource constrained small-scale farmers have experience serious losses (Chimweta *et al*., 2020) [4]. There have also been concerns about the environmental and health impacts of some synthetic chemical pesticides. The maize producers have resorted to using various types of synthetic pesticides to decrease economic injury levels. Additionally, the pest is reported to be highly resistant to many common pesticides on the market (Horikoshi *et al*., 2016; Wan *et al*., 2021; Bird *et al*., 2022; Prasanna *et al*., 2022) [11, 23, 2, 22]. There is, therefore, need to explore the use of biopesticides, which are generally considered safer to the environment and human health. Biopesticide research and development efforts for fall armyworm control have however experienced several challenges including the many strains of the pest that exhibit various levels of response to synthetic formulations and biopsied. For example, virus formulations are affected by, *inter alia*, prevailing climatic conditions; entomopathogenic fungal isolates

Identified thus far offer limited control; commercial entomopathogenic nematode applications have not yet been developed especially in Africa, and efficacy of *Bacillus thuringiensis* (*Bt)* formulations and botanicals against fall armyworm have not yet been determined. Furthermore, *S. frugiperda* has developed resistance to *Cry* and *Vip3A* proteins of *Bt* expressed in transgenic maize (Horikoshi *et al*., 2016; Santos-Amaya *et al*., 2022) [11, 18]. With the fall armyworm likely to remain a significant agricultural pest across many regions for the foreseeable future, it is essential to develop an effective, coordinated, but flexible approach to manage the pest. An Integrated Pest management approach which would include the use of biopesticides provides a useful framework to achieve these goals

It is upon this background that biopesticides which are largely environmentally benign be adopted in an integrated *S. frugiperda* management program.

Agriguard®, AgriX365 is one of the many botanical biopesticides that has a potential to supress *S. frugiperda* infestations to below injury level. The constituents of this formulation include oils of clove, peppermint, rosemary, sesame, thyme and lemon grass (Table 1). It acts on the amino acids in insect proteins. AgriX365 works by breaking down the bonds in non-salt bonded cell wall proteins leaving the salt bonded proteins unharmed (AgriGuard Biosystems Report, 2022) [1]. Surprisingly, this process is selective via the salt marker within the protein itself. However, the response of *S. frugiperda* to AgriX365 has not been tested to determine the LD_{50}.

The aim of the experiment was to establish the LD_{50} for AgriX365, the amount of AgriX365 given at once that will kill 50% of the *S. frugiperda,* a measure of the acute toxicity of the botanical pesticide (Segreti and Munson, 1981; Raj *et al*., 2013) [20, 17].

Table 1: Active ingredients in Agri Guard AgriX365- a biopesticide

Active ingredients	Composition (%)
Clove oil	2.5
Peppermint oil	2.2
Rosemary oil	1.9
Sesame oil	1.9
Thyme oil	1.9
Lemon grass oil	1.35
Sodium Lauryl Sulphate	0.29

Methodology

FAW were collected from an untreated maize field at Benwell Farm (31°11'59" S; 17°12'45" E) in Mashonaland Central, Zimbabwe. The FAW were allowed to settle for 24 hours while being fed with excised maize leaves and kennel until pupation under the following environmental conditions (25°C, 70±5% RH and 10:14 L: D photoperiod). The pupae were then transferred into cage under same environmental conditions until adult eclosion. The adults were allowed to mate and oviposit while being fed with 5% honey: water solution in a cage with maize plants at v4 stage. The eggs were laid, allowed to hatch and larvae allowed to develop to 3rd instar which were used in the experiment. A total of 400 3rd instar FAW larvae were used in the experiment comprising four treatments.

Treatment 1(T1)- Control sprayed with water only

Treatment 2 (T2)- 0.5x the standard field rate of AgriX365 (0.5ml/L water) (500ppm).

Treatment 3 (T3) - the standard/recommended field application rate (1ml/L water) (1000ppm).

Treatment 4 (T4) - double the standard application rate (2ml/L water) (2000ppm).

For each treatment, 20 FAW larvae were used in which they were topically (sprayed) treated with a given concentration of AgriX365. The experiment was replicated five times in a complete randomised design at room temperature (25°C, 70±5% RH and 10:14 L: D photoperiod.

A fresh excised piece of tender maize leaf (about 5cm x5cm) was placed in each of the large ventilated sterile petri dishes (10cm diameter). The larvae were then individually) placed in the petri dishes after which they were sprayed with the appropriate treatment. They were then covered and left to feed. The larvae were provided with fresh treated leaves every 24 hours. The larvae were checked for mortality every 24 hours for 72 hours or until most of the treated larvae were dead or moribund.

Data analysis

The mortality of FAW larvae due to AgriX365 was assessed using a logistic function Probit, *P*, in Exel. The proportion, *P*, was established by the point at which the proportion reached the 0, 5 mark and because of the large uncertainties, where only a few FAW larvae died in Treatment 1 (control), only values for T2-T4 were used. However, a correction factor (Equation **1**) was used to account for FAW larvae that could have died independent of AgriX365 treatment.

Correction factor = Observed Proportion-Control Proportion/1-Contrtol Proportion [1]

The LD_{50} was given by the intercept of the best-fit line as it crossed the *x*- axis from which the LD_{50} is calculated for AgriX365.

Abbott's formula (Abbot 1925), equation **2**, was used to correct for natural mortality.

$$\text{Corrected \% Mortality} = \left(1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment}}\right) * 100 \quad [2]$$

Where: n = Insect population, T = treated, Co = control.

An analysis of variance of the mean mortality of *S. frugiperda* larvae was assessed using Rafter correcting for natural mortality using Abbot's correction formula [2].

Results

There was a relatively dose-dependent response in mortality rates of *S. frugiperda* from 0.5ml/L to 2ml/L of AgriX365. The logistic function Probit, *P*, calculation indicated an LD_{50} =1.25ml/L (1250 ppm); y=0.5998*x*+4.2749; R^2=0.9979) of the standard or recommended field rate for AgriX365 (Figure 1).

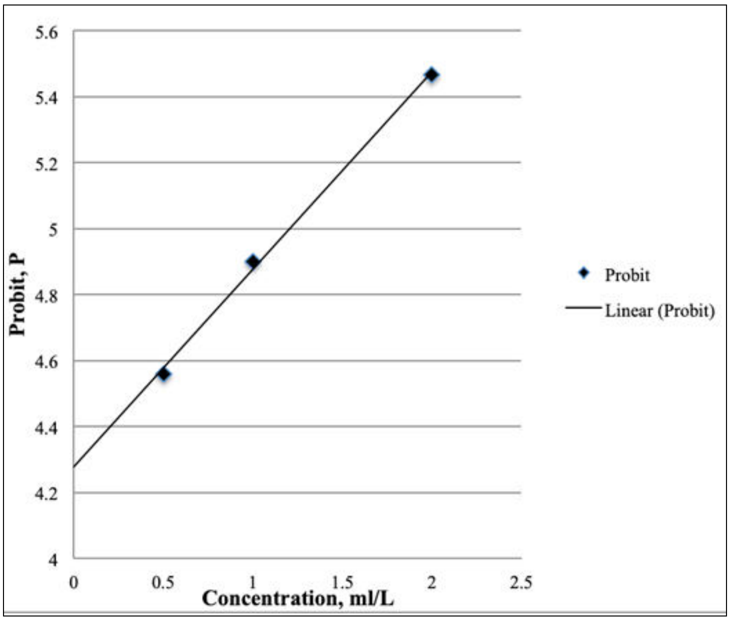

Fig1: Determination of LD_{50} for AgriX365 using Probit function. Arrow indicates a dose (1.25ml/L (1250ppm) where 50% of 3rd instar *S. frugiperda* died.

There was a high significant difference between mean per cent mortality between treated *S. frugiperda* larvae and the untreated ones ($p<0.001$; df=3; $F=36.91$) and between AgriX365 treatments ($p<0.01$) (Figure 2).

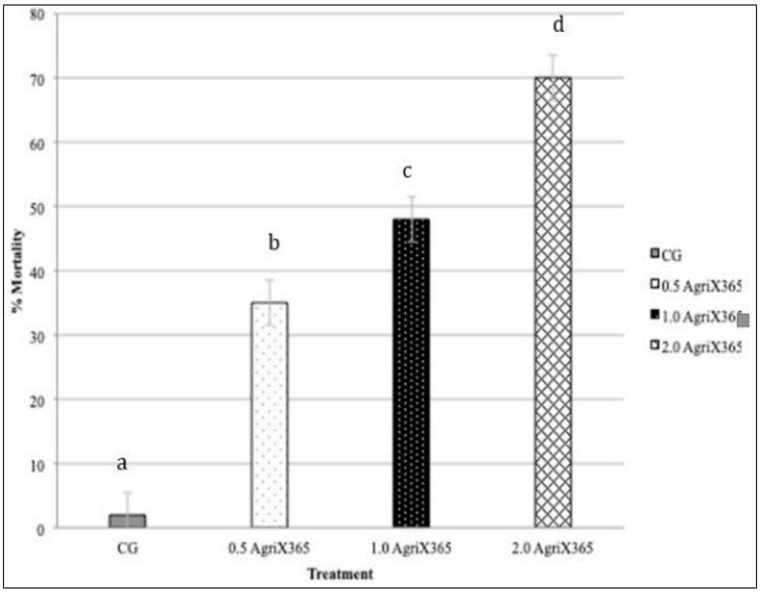

Fig 2: Mean per cent mortality of *S. frugiperda* larvae (3rd instar) subjected to various doses of a botanical pesticide, AgriX365. The control group, CG, is included.

Discussion

The mode of action of AgriX365 ensures that on contact, the insect proteins in the exoskeleton or hydrostatic skeleton in the case of larvae, are disrupted leading to gradual death of the insect pest. An increase in the dose elicits increased effect on the amino acid proteins. However up to a certain dose, the median lethal dose can be achieved. A good dose is that which will kill fifty per cent of the target pest in at least 72 hours after application. AgriX365 has a large LD_{50} value (1250ppm) in the tested *S. frugiperda* and hence is not hazardous and does not pose environmental or health risks. In general, the smaller the LD_{50} value the more toxic the substance and the large the LD50 value, the lower the toxicity (British Columbia Report, 2022). Most pesticides used in the control of *S. frugiperda* are effective but have low LD_{50} values (Hardke, 2011; Hong, 2020; Shareef *et al*., 2021) [9, 10, 21].

In modern agriculture, pesticides play a key role by providing dependable, persistent, and relatively complete control against harmful pests with limited labour and relatively low cost. Crop yield have been increased through use of pesticides that suppress pest populations effectively and quickly. However, these agrochemicals considerably pollute the environment and pose undesirable effects on non-target organisms (Gill and Garg, 2014; Sharma *et al*., 2019; Riyaz, 2021) [7, 15, 22]. Injudicious use of pesticides in agriculture has serious environmental and consumer health hazards. Most synthetic pesticides have long residual action affect non- target species (Thompson, 1996; Shafiq-ur-Rehman, 2006) [16, 14] and there are reports on unpleasant consumer health complications.

Unlike synthetic pesticides, biopesticides, also known as biorational pesticides, do not cause total eradication of the existing pest population and other organisms that are not the target of treatment. This helps keep the ecosystem in health equilibrium.

Conclusion

Therefore, the standard application rate of AgriX365 is 1.25ml/L, for 50% of 3rd instar *S. frugiperda* larvae to die. However, this quantity may be increased slightly when applied in open field. The impact of AgriX365 on non-target species, for example, earwigs (Dermaptera: Forficulidae) which predate on *S. frugiperda* need to be studied as well as possibility of tolerance by *S. frugiperda* with continued exposure.

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Competing interests

The authors declare no competing interests.

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