Research Article

LABORATORY BIOASSAY OF FALL ARMYWORM (*Spodoptera frugiperda*) LARVA USING VARIOUS INSECTICIDES

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Received date: 12 December 2021, Accepted date: 23 March 2022

ABSTRACT

Fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith, is a polyphagous invasive pest that seriously affects the maize crop. Various insecticides such as Spinosad, Chlorantraniliprole, Imidacloprid, Emamectin benzoate, Spinetoram and Neem-based insecticides are recommended to control this pest. However, their efficacy is not well studied in Nepal. Hence, a study was performed to evaluate the efficacy of the above-mentioned insecticides for FAW management in laboratory experiments. Seven different treatments (six insecticides such as Spinosad 45% SC, Chlorantraniliprole 18.5% SC, Imidacloprid 17.8% SL, Emamectin benzoate 5% SC, Spinetoram 11.7% SC, Neem-based pesticide (Azadirachtin 1500 ppm) and control (water spray) were evaluated in three replicated CRD design. Spinosad and Spinetoram were found effective for the FAW mortality in which > 50% mortality of the larva was obtained in twelve hours and > 90 % mortality in twenty-four hours. Likewise, Emamectin benzoate and Chlorantraniliprole also caused > 90% mortality within twenty-four hours. Azadirachtin and Imidacloprid were not as effective as other pesticides that caused only 17% larval mortality in the first twelve hours and 68% mortality in sixty hours. Similarly, Imidacloprid caused 8% larval mortality in twelve hours and 59% mortality in sixty hours. There was no mortality in water spray (control). This information gives an idea of all pesticides are not equally effective and efficient. Such information's are important to the farmers to select the right insecticides for the control of FAW in maize crops.

Keywords: Fall armyworm, bioassay, pesticides, efficacy, mortality, time interval

INTRODUCTION

Fall armyworm (*Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) is a major pest of maize crop in all maize growing districts of Nepal. It is a polyphagous and voracious pest. Fall armyworm infestation has been reported on more than 300 species of plants (Montezano, 2018). The larval stage of FAW feeds on maize whorls and cobs. Adults are active fliers and can fly about 150 km in a day (Johnson, 1987). It has been reported that fall armyworm causes more than $13 billion (USD) in crop losses per annum in Sub-Saharan Africa, resulting in food insecurity for millions of marginalized and poor farmers (Day et al., 2017). FAW has spread to 47 countries in Africa, 17 countries in Asia and 105 countries in the world (Hruska, 2019). On May 9, 2019, this pest was observed for the first time in Nawalpur district of Nepal (Bajracharya et al., 2019), causing 20-35% maize losses. The Plant Quarantine and Pesticide Management Centre of the Ministry of Agriculture and Livestock Development reported that out of 77 districts in Nepal, FAW infestation occurred in 65 districts (Onlinekhaber, 2021). In Nepal, a total of 956,477 ha of land is covered by maize area with the production of 2,713,635 tons (MOAD, 2020). The loss of maize caused by FAW is very high accounting for 30 - 70% yield loss (MOALD, 2019).

Pesticide application is one of the major FAW management practices in developing countries. However, these practices are not sustainable and have the potential to cause deleterious effects on human health, the environment, and biodiversity. Furthermore, the use of chemical insecticides results in pesticide resistance development in many insect pests and effects on non-target organisms such as pollinators and natural enemies. Additionally, pesticide costs are greater than other management approaches and are expensive for small-scale farmers. Various pesticides such as Spinetoram Spinosad, Chlorantraniliprole, Azadirachtin etc are recommended for the control of this pest (Bhusal & Bhattarai, 2019; Hardke et al., 2014). Their efficiency has not been studied well before the application in open field conditions. These practices further accelerate the unintentional loss of pesticides and money which increases the input cost of farmers. Hence, a laboratory bioassay was conducted to select an effective and efficient insecticide for the FAW management, which is necessary to select an appropriate and efficient pesticide and save pesticide costs for farmers.

MATERIALS AND METHODS

A bioassay/experiment on the fall armyworm (FAW) was conducted in the Entomology laboratory of Agriculture and Forestry University (AFU) in July 2021. A total of seven different treatments including control (water spray) with three replications were taken for the study and observed the mortality of fifth instar FAW larva at different time intervals.

Each treatment (pesticide) was prepared according to the required dose (Table 1). Fifteen individual Petri dishes (9 cm diameter, 1.5 cm height) were taken for each treatment and the Petri dishes were coded from 1-15 and randomized into three replications with five Petri dishes in each replication. This was done for all the treatments. Uniform sized maize leaves (7*4 cm^2) (4^{th} to 5^{th} leaf of knee height stage plant) from the pesticide-free field were used to feed larvae. The leaves were dipped in the respective insecticide solution for 15 seconds. For the control treatment, tap water was used, gently dipped in a beaker and kept for about a minute for air drying. Then the leaves were transferred to the Petri dishes of sizes 9 cm in diameter, 1.5 cm in height.

Table 1. Treatments used in bioassay experiments

S.N.	Treatments	Trade name	Level	Dose/lit water
1	Azadirachtin 1500 ppm	Gorkha Bio-Neem	Green	4.0 ml
2	Emamectin benzoate 5% SG	G- Super	Yellow	0.4 gm
3	Spinosad 45% SC	Tracer	Blue	0.3 ml
4	Chlorantraniliprole 18.5% SC	Allcora	Green	0.4 ml
5	Spinetoram 11.7% SC	Delegate	Green	0.3 ml
6	Imidacloprid 17.8% SL	Rajmida	Yellow	1.0 ml
7	Water spray	Tap Water	-	Gently dipped

The second-generation fifth instar larvae were taken from the laboratory rearing FAW colony and kept individually in the Petri dish (9 cm diameter, 1.5 cm height) and kept for 24 hours for starvation. Insecticide dipped leaves were kept inside Petri dish and covered by a lid (9.4 cm diameter and 1 cm height). The mortality of the larva was observed at 12, 24, 36, 48, and 60 hours and this was continued for four days. The experiment was arranged in a completely randomized design (CRD) and a one-way analysis of variance was performed by using GenDisc4 (GenStat). The means were separated by using Tukey's significant difference test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

There was a significant difference among the synthetic pesticides in the larval mortality of FAW (Table 2). More than 50% mortality of FAW larva was observed in twelve hours by Spinosad and Spinetoram pesticides whereas only 8% mortality was observed by Imidacloprid and 17% mortality by Azadirachtin. Spinosad has been found as the most effective pesticide with the highest mortality of FAW larva within a very short time as explained by Cook et al. (2004).

Table 2. Mean percentage (± SEM) of mortality of FAW larvae at 12, 24, 36, 48 and 60 hours after application of pesticides in laboratory bioassay

Treatments	Percent larval mortality ± SEM at different time intervals				
	12h	**24h**	**36h**	**48h**	**60h**
Azadirachtin1500 ppm	17.71±8.86abc	30.79±4.22b	63.85±13.07b	63.85±13.07ab	68.07±11.56ab
Emamectin benzoate 5% SG	38.86±6.99abc	90.00±0.00a	90.00±0.00a	90.00±0.00a	90.00±0.00a
Spinosad 45% SC	51.14±6.99ab	90.00±0.00a	90.00±0.00a	90.00±0.00a	90.00±0.00a
Chlorantraniliprole 18.5% SC	30.79±4.22abc	90.00±0.00a	90.00±0.00a	90.00±0.00a	90.00±0.00a
Spinetoram 11.7% SC	56.15±16.92a	90.00±0.00a	90±4.22 a	90.00±0.00a	90.00±0.00a
Imidacloprid 17.8% SL	8.86±8.86bc	38.86±6.98b	59.21±4.22 b	59.21±4.22b	59.21±4.22b
Water spray	0.00±0.00d	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Grand Mean	29.10	61.40	69.00	69.00	69.60
CV %	52.00	8.70	12.70	12.70	11.10
LSD (5%)	26.89	3.30	5.60	5.60	5.30
P-value	0.004*	<0.001**	<0.001**	<0.001**	<0.001**

Note: CV: Coefficient of Variation; **: Significance at 1% (p<0.001); *: Significance at 5% (p<0.05); LSD: Least Significant Difference; Values with the same letters in a column are not significantly different at 5% by Tukey's significant difference test; Values are in percentage, Sem (±) indicates standard error of mean percentage (% value ± Standard error of mean percentage); h= hour

Similar studies have been conducted around the world including China, Africa, Brazil and India to test the efficacy of pesticides in field and laboratory conditions to control FAW, and reported that FAW is susceptible to synthetic pesticides, however, Spinosad, Chlorantraniliprole and Emamectin benzoate causes the highest mortality compared to other synthetic pesticides (Idrees et al., 2022). Continuous application of such pesticides in the field may increase resistance against many categories of pests including FAW (Osae et al., 2022). The study conducted by Zhao et al. (2020) suggested that *S. frugiperda* are resistant to Lamda-cyhalothrin due to the continuous application of pesticide in the maize field. Similarly, there are other insects such as diamondback moth and two-spotted spider mite which are resistant to abamectin (Kwan et al., 2010). The time of exposure, as well as concentration, affects the mortality rate as well as toxicity of the insects including FAW (Cook, Leonard & Gore, 2004).

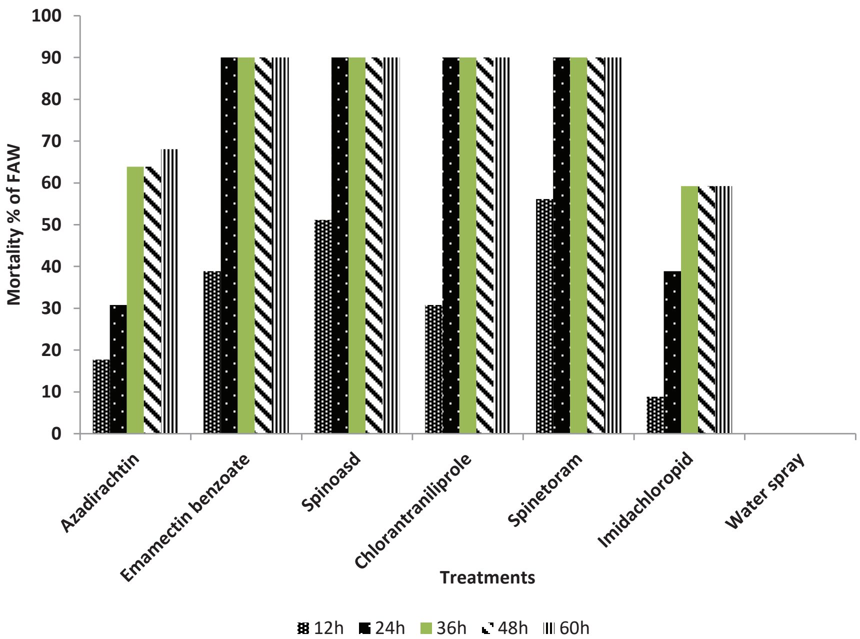

Figure 1. Percentage mortality of FAW at different time intervals caused by various bioassay materials

The Chlorantraniliprole and Emamectin benzoate showed a mortality of 31% and 39%, respectively within 12 hours of FAW larval release. Sisay et al. (2019) reported that Imidacloprid caused 40% mortality in 24 hours and 70% mortality in 72 hours. In twenty-four hours of the treatment application, all four pesticides, i.e. Spinosad, Spinetoram, Chlorantraniliprole and Emamectin benzoate caused more than 90% larval mortality, showing their high effectiveness for the FAW. Similar results were also reported by Sisay et al. (2019) and revealed that Spinosad and Spinetoram caused more than 90% mortality in a laboratory experiment.

A similar pattern of higher mortality of FAW larvae within a very short time by Spinosad and Spinetoram was observed in an experiment conducted by Belay et al. (2012). Similarly, Chlorantraniliprole caused mortality of 30% in the first twelve hours and more than 90% within twenty-four hours in this experiment which is similar to the results obtained by Sisay et al. (2019), Hardke et al. (2011) and Thrash et al. (2013).

Azadirachtin and Imidacloprid caused only 31% and 39% larval mortality within twenty-four hours. There was no mortality of larvae in control (water spray). There were no significant differences in twenty-four, thirty-six and seventy-two hours of treatment application in mortality of the FAW larvae in all the treatments. After sixty hours of the experiment setup, 68% larval mortality was observed in Azadirachtin and 59% mortality of larvae in Imidacloprid. In some parts of the world, farmers have used Imidacloprid which is less effective than the Azadirachtin. Hence, in such a situation, neem-based pesticides can be recommended to keep the pest below the damage threshold level. Neem-based pesticides are safer and more eco-friendly compared to Imidacloprid (Mordue et al., 2010). But dose and frequency of neem-based pesticides can influence the percentage of FAW mortality. Likewise, Emamectin benzoate and Chlorantraniliprole also caused more than 90% mortality of the FAW within 24 hours though their effectiveness in the early twelve hours was quite lower as compared to Spinosad and Spinetoram.

This study was conducted in a laboratory experiment in Chitwan condition but the effectiveness of such pesticides in other ecological zones may differ. However, a similar study conducted in similar agroecological zones by Bajracharya, Bhat and Sharma (2020) revealed similar results. According to them, the effectiveness of Spinosad, Chlorantraniliprole, and Emamectin benzoate was more promising as compared to Azadirachtin and Imidacloprid in open field conditions. The stage of FAW larvae used in the bioassay experiment also affects the mortality rate. Early instars can be more susceptible to pesticides compared to the later stages. Chemical managements are popular for the FAW but the use of these synthetic pesticides involves high cost, potential environmental and human health issues, and pest related problems (Tudi et al., 2021; Muratet et al., 2015; Choudhary et al., 2018). In Nepal, there are five common pesticides registered by the Plant Quarantine and Pesticide Management Centre (PQPMC), such pesticides are Azadirachtin 1500 ppm @ 5 ml/liter, Spinetoram 11.7 SC @ 0.5 ml/liter of water, Chlorantraniliprole 18.5% SC @ 0.4 ml/liter of water, Spinosad 45%SC @ 0.3 ml/liter of water, Emamectin benzoate 5% SG @ 0.4 g/liter of water (MoALD, 2019). However, the current use practices of pesticides are not safe for human health and the environment (Bateman et al., 2021). This study provides preliminary information on efficiency of pesticides before testing them in field conditions. Accordingly, effective, alternative and safe management strategies are recommended for sustainable FAW management.

CONCLUSION

Fall armyworm (*S. frugiperda*) is an invasive lepidopteran pest of maize crops. This pest was formally noticed in Africa in 2016 and in Asia in 2018. For the first time, this pest was identified in Nawalpur, Nepal in 2019. The invasive status of this pest was first officially declared by NPPO Nepal on 12 August 2019. Being an invasive status, the Government of Nepal immediately recommended five insecticides for immediate control and to prevent further spreading. These pesticides are Azadirachtin 1500 ppm, Spinetoram 11.7 SC, Chlorantraniliprole 18.5% SC, Spinosad 45% SC, Emamectin benzoate 5% SG. The effectiveness of such pesticides in 2019 was not tested in the laboratory and field in Nepal. Later, these pesticides were tested in the maize field by the researchers in research stations and students in field crops. The laboratory bioassay was not carried out to see the laboratory response of these common pesticides for the FAW larvae. Hence, this study aimed to see the effect of such common pesticides on FAW management in laboratory conditions.

Results showed that all these pesticides are not equally effective for FAW. Based on the laboratory bioassay, Spinosad, Spinetoram, Chlorantraniliprole, and Emamectine benzoate are categorized in the effective group and the other two Imidacloprid and Azadirachtin are grouped into non-effective category. Among the first group, Spinosad and Spinetoram caused more than 50% mortality in twelve hours and > 90% mortality in twenty-four hours. Chlorantraniliprole and Emamectin benzoate caused < 50 % larval mortality in 12 hours and > 90% mortality in twenty-four hours. Similarly, only 17% of mortality was caused by Azadirachtin in 12 hours and 70% in sixty hours. The least effective synthetic pesticide Imidacloprid caused only 8% mortality in 12 hours and less than 60% mortality in sixty hours. Such research findings indicate that all insecticides are not equally effective. First, it is necessary to test their efficacy in laboratory conditions before using them in field conditions. The research findings suggest that farmers should be serious when buying pesticides from agro-vets in order to save cost and amount of pesticide spray in maize farms.

ACKNOWLDGEMENTS

The authors are grateful to the Directorate of Research and Extension (DOREX) of Agriculture and Forestry University, the University Grants Commission Nepal, Department of Entomology/AFU and the National Maize Research Programme (NMRP) for the financial and technical supports.

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