ENTOMOLOGY

Efficacy evaluation of some bio-insecticides against green leafhopper (*Amrasca biguttula biguttula Ishida*) infesting brinjal

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Abstract

In Bangladesh, brinjal (*Solanum melongena* L.) is an important vegetable crop due to its year-round cultivation, high demand for consumption, and nutritional value. The main obstacle to the successful cultivation and production of brinjal is insect pests. The current study was carried out in the winter, when green leafhoppers (*Amrasca biguttula biguttula*), one type of sucking insect pest, are most prevalent. Early in the vegetative stage, an infestation of green leafhoppers (GLH) was noted; as the canopy size increased, so did their numbers. The consumption of brinjal with its peel poses a health risk due to possible contamination with toxic chemical insecticides. We assess a few non-toxic or minimally toxic bio-insecticides against GLH in order to tackle this crucial problem. Fizimite, one of the bio-insecticides, was found to be effective against GLH in a sodium lauryl ether sulfate preparation. Fizimite decreased the amount of GLH in the plant by 85.8%, the amount of leaf infestation by 77.84%, and the amount of GLH-infected brinjal leaf abundance by 54.34%. But Voliam Flexi, a chemical control, was also discovered to be successful against GLH. Fizimite may therefore be a non-toxic, bio-rational substitute for Voliam Flexi, a synthetic, toxic medication used to control GLH.

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Key words: brinjal (*Leucinodes orbonalis* L.), green leafhopper (*Amrasca biguttula biguttula*), bio-insecticides, toxicity, leaf infestation.

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Introduction

Brinjal (*Solanum melongena* L.), also known as eggplant or aubergine is a vitamin, mineral, phenol, and antioxidant-rich vegetable (Gurbuz *et al.*, 2018). This vegetable is cultivated throughout the world because of heat insensitivity, and genetic or morphological diversity (Chapman, 2020) with a production of 55,197,878 tons from a 1,847,787 ha area (FAO, 2019). However, insect pests are the major impediment to the successful cultivation and production of brinjal (Mollah *et al.*, 2022a). Insect infestation starts at the seedling stage and continues up to the final harvest. About 140 species of insect and arachnid pests are reported to infest brinjal throughout the world (Sharma and Tayde, 2017). In Bangladesh, green leafhopper (*Amrasca biguttula biguttula*), aphid (*Aphis gossypii*), whitefly (*Bemisia tabaci*), thrips (*Thrips tabaci*), shoot and fruit borer (*Leucinodes orbonalis* G.), and from arachnid, red spider mite (*Tetranychus macfurlanei*) are most prevalent (Dutta *et al.*, 2017). Among these, brinjal shoot and fruit borer (BSFB), leafhopper, whitefly, and mealy bugs are most common, prevalent, and destructive in the summer season (Kapil *et al.*, 2022; Mollah *et al.*, 2023) while aphids are most common in both winter and summer seasons in brinjal and leguminous vegetables causing low yield (Mollah, 2022; Mollah *et al.*, 2017a; Mollah *et al.*, 2013a; 2013b). Both adults and nymphs of leafhoppers and other leaf suckers cause serious damage to leave by sucking cell sap from the lower surface of the leaves causing chlorosis and necrotic spots. Finally, the entire plant turns brown and shows burn symptoms and ultimately the leaves droop off. The indirect effect of leafhoppers includes honeydew secretion that facilitates fungal growth and interrupts photosynthesis (Bosquee *et al.*, 2018).

Although chemical control by synthetic insecticides is mostly practiced for managing leafhoppers and other sucking pests, their overuse causes insecticide resistance and the outbreak of secondary pests (Bass *et al.*, 2014). Several detrimental effects including mortality of non-target organisms (Mollah *et al.*, 2017b), beneficial predators (Mollah *et al.*, 2012a) or parasitoids (Mollah and Khatun, 2023), and insecticidal residues on fruits (Blair *et al.*, 2015) are also observed. Therefore, the introduction of non-hazardous, plant-based, or microbial-origin bio-insecticides is very essential. Additionally, these are mostly effective, safe for non-target organisms and natural enemies, and target pest-specific, and ecologically sound solutions to pest problems (Mollah *et al.*, 2022b). Some commercially formulated microbial or pathogenic bio-insecticides were found effective against the major insect pests of brinjal, brinjal shoot, and fruit borer (Mollah *et al.*, 2023) and the sucking insect pests (Gayathri and Geetha, 2019). The efficacy of pathogenic or microbial insecticides might come from the combined effect of toxicity (Tobias *et al.*, 2018; Mollah *et al.*, 2020a), insect host immunity suppression (Mollah *et al.*, 2020b; Mollah and Kim, 2020), inhibition of immune mediator secretion (Hasan *et al.*, 2019, Mollah, 2023) and bind with immune proteins (Mollah, 2024; Mollah *et al.*, 2021). Some botanical insecticides were found effective against the bean pod borer (Mollah *et al.*, 2012b) and sucking insect pests including aphids of country bean (Mollah *et al.*, 2013b) and green leafhoppers of brinjal (Kunbhar *et al.*, 2018). However, little or no information is available about the efficacy of commercially available bio-insecticides against green leafhoppers in the brinjal field. Therefore, the present study aimed to evaluate some commercially formulated bio-insecticides against green leafhoppers, infesting brinjal plants under field conditions. For this, some commercially available bio-insecticides were applied in brinjal fields grown during the winter season.

Materials and Methods

Location, soil characteristics, and land preparation

This study was conducted during the winter season (November 2021 to March 2022) at the Bangladesh Institute of Nuclear Agriculture substation, Barisal, Bangladesh to evaluate the performance of some microbial and plant-origin bio-insecticides against green leafhoppers (GLH) infesting brinjal plant. This area possesses calcareous, alluvium, and calcareous brown floodplain soil belonging to the Agro-Ecological Zone of Active Ganges Floodplain (AEZ 10) (Khatun and Mollah, 2023). Soils are characterized by low organic matter and mildly alkaline in reaction. The soil has a medium fertility level with a nitrogen deficiency (N), pH ranging from 5.8-6.5, and cation exchange capacity is 25.58 with silty clay loam textured soil. The study area is 2 meters above sea level and situated at 22.8162°N latitude and 90.3137°E longitude (Khatun and Mollah, 2023). The climate is characterized by the alternate hot and cold seasons. Repeated tillage was provided to ensure good tilth condition of the soil and fertilized properly for successful crop production.

Experimental design, planting, and treatment application

A randomized complete block design with three replications was adopted for this study. Good quality and healthy seedings were planted maintaining 0.8 m plant-to-plant distance and 0.8 m row-to-row distance. All the intercultural operations including irrigation, weeding, mulching, and fertilizations were performed whenever needed. The treatment includes some bio-insecticides as: T1: untreated control, T2: Biomax M 1.2 EC (Abamectin) @ 1.0 mL L^{-1} of water, T3: Fizimite 20% (Sodium Lauryl Ether Sulphate) @ 1.0 mL L^{-1} of water, T4: Fytomax 0.1% (Azadirachtin) @ 5.0 mL L^{-1} of water, T5: Spinomax (*Saccharopolyspora spinosa* + Btk broth) @ 2.0 mL L^{-1} of water, T6: Fytoclean 40% (Potassium salt of fatty acid) @ 5.0 mL L^{-1} of water, T7: Volium Flexi 300 SC (Chlorantraniliprole + Thiamethoxam) @ 0.5 mL L^{-1} of water. All the bio-insecticides were purchased from Russell IPM Bangladesh Limited and the chemical insecticide Voliam Flexi 300 SC was collected from the local market, and their details are provided in Table 1. Insecticides were applied at noon to avoid the toxic effect on pollinators as honeybees and wasps are mostly active in the afternoon from 1:00 to 4:00 PM in the crop field. Application dose was followed according to the previous study (Mollah *et al.*, 2022a; Mollah *et al.*, 2023) and the manufacturer's description. To prepare the spray mixture, water was directly added to the sprayer tank already containing bio-insecticides and mixed well for convenient foliar spray. A measuring cylinder was used to confirm the desired concentration of the bio-insecticides. A hand sprayer (SeeSa®) was used to apply the insecticide mixture and it was applied at the rate of 300-700 mL $plant^{-1}$ depending on the canopy size and growth stage of the plants. Both sides of the leaves were sprayed well with the spray mixture. Precautions were taken to avoid the contamination of insecticides by preventing the drifting of the spray mixture. Spraying was started at the early flowering stage and continued up to the last harvest with an interval of 10 days throughout the flowering and fruiting stage. After the application of an insecticide, the sprayer was cleaned very well by repeated washing with clean water, then another spray mixture was prepared.

Data collection and presentation

Data collection started at the early vegetative stage before bio-insecticide application to observe the pattern of GLH infestation. At this stage, data was collected on each 4th day on the number of GLH per plant. For this, all the leaves of all the plants were checked. After seven observations, at the early flowering stage, treatments were applied. All the leaves of all the treated plants were observed twice (5th and 10th day) between the spray interval of 10 days to record the effect of insecticides on the GLH population. At this stage, data collection was conducted on the number of GLH in each plant, the number of infested and healthy leaves in the infested plant to work out the percent leaf infestation, and the number of GLH in each infested leaf to show the abundance of green leafhoppers in the field. The collected data were processed, analyzed, and applied for graph preparation. The percent leaf infestation and percent reduction of leaf infestation over control were found using the formulae described in a previous study by Mollah and Hassan (2023):

$$\text{Percent leaf infestation} = \frac{\text{Number of infested leaves}}{\text{Total number of leaves}} \times 100$$

$$\text{Percent reduction over control} = \frac{\text{Mean value of the treatment} - \text{mean value of the control}}{\text{Mean value of the control}} \times 100$$

Statistical analysis and presentation of data

For processing, calculating, and arranging the collected raw data, Microsoft Excel was used. PROG GLM in the SAS program (SAS, 2002) was used for the analysis of variance (ANOVA) for all the continuous variables data. The least significant difference test at

0.05 level of type I error was applied to compare the means. Every treatment was replicated thrice in three individual plants. Each bar represents the mean value of the replications and error bars represent the standard deviation. Graphical data were prepared using Sigma Plot 12.5 software.

Results

Infestation and pattern of infestation in brinjal field at the vegetative stage

The present study found that GLH infested the lower surface of the brinjal leaf (Figure 1A) and the infestation starts from the early vegetative stage. In the infested plants, GLH abundance increased in most cases with the increase in plant age and canopy size although exceptions were found in some cases (Figure 1B). The abundance or number of GLH was different among the plants grouped under different treatments. The maximum number of GLH was observed in T6 which was followed by T7, T4, T5, T3, and T2 while the least was in T1 (Figure 1B).

Effect of bio-insecticides on GLH infestation in brinjal plant

Application of bio-insecticides significantly (F=120.1; df=6, 14; P<0.001) affects the GLH infestation in the brinjal plant. The highest GLH individual was recorded from Fytoclean (7.44 GLH/plant) treated plants which were statistically similar to Biomax M (6.77 GLH/plant), and Fytomax (6.44 GLH/plant) (Figure 2A). In contrast, the lowest GLH individual was observed in Fizimite (2.66 GLH/lant) treated plants (Figure 2A). Moreover, very few GLH (0.33 GLH/plant) were observed in the chemical control Volium flexi-treated plants. However, the maximum number of GLH was recorded from untreated control plants (18.77 GLH/plant) (Figure 2A). Thus, the maximum reduction of GLH over untreated control was achieved from Fizimite (85.88%) which was followed by Spinomax (76.33%), Fytomax (65.68%), and Biomax (63.91%) while the minimum was obtained from Fytoclean (60.36%) of the applied bio-insecticides (Figure 2B). However, the chemical insecticide Volium flexi provided a 98.23% reduction of GLH over untreated control (Figure 2B).

Similarly, bio-insecticides have a significant (F=219.6; df=6, 14; P<0.0001) effect on the reduction of leaf infestation by GLH (Figure 3). Among the bio-insecticides, the lowest infestation in leaf by GLH was obtained from the Fizimite (6.27%) application which was statistically different but followed by Spinomax (8.46%), Fytoclean (8.63%), and Biomax (12.17%) while the highest was observed in Fytomax (14.78%) treated plants. Moreover, the chemical insecticide Volium Flexi also confirmed very low (1.55%) leaf infestation by GLH. However, the untreated control plants ensured maximum leaf infestation by GLH (28.32%) (Figure 3A). Therefore, the maximum reduction of leaf infestation over untreated control was achieved from Fizimite (77.84%) application which was followed by Spinomax (70.10%), Fytoclean (69.54%), and Biomax (57.06%) while the lowest reduction of leaf infestation was achieved from Fytomax (47.8%) application of the bio-insecticides (Figure 3B). However, the chemical control Voliam flexi provided a 94.52% reduction of leaf infestation by GLH (Figure 3B).

Bio-insecticides significantly (F=27.49; df=6, 14; P<0.001) control the abundance of GLH in the infested leaf also (Figure 4). Among these, Fizimite (1.29 GLH/infested leaf) and Fytoclean (1.27

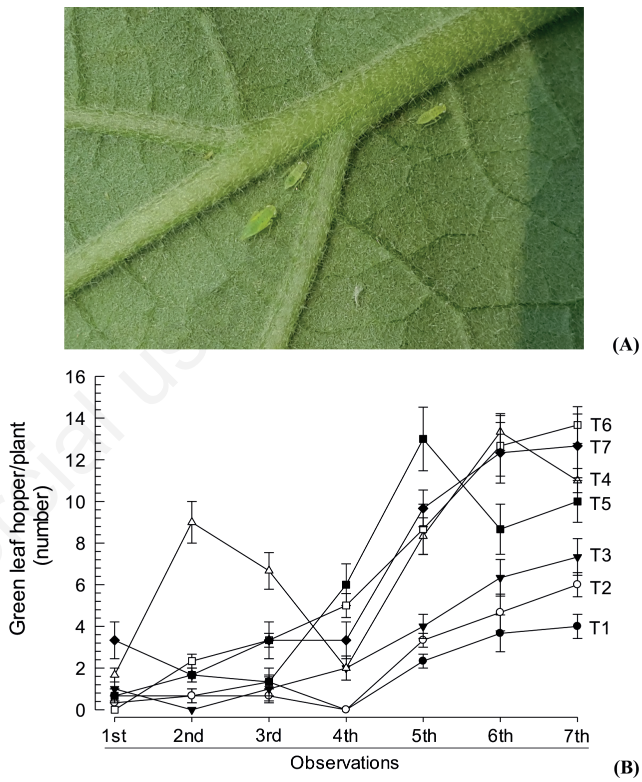

Figure 1. Infestation of green leafhoppers in brinjal plant. (A) Green leafhopper-infested brinjal leaf; (B) pattern of leaf infestation. Plants were randomly divided for treatments. Each treatment consists of three replications and each plant was considered a replication. The number of infested and healthy leaves was counted for leaf infestation calculation. Different letters above the error bar denote significant differences (P<0.05, least significant difference test) among the treatments.

Table 1. Different treatments used in this study with their pieces of information.

Treatments	Active ingredient	Suppliers	Dose
T1 = Water	N/A	N/A	N/A
T2 = Biomax M 1.2 EC	Abamectin	Russell IPM BD Ltd.	1 mL L^{-1} water
T3 = Fizimite 20%	Sodium laurylether sulphate	Russell IPM BD Ltd.	1 mL L^{-1} water
T4 = Fytomax 0.1%	Azadirachtin	Russell IPM BD Ltd.	5 mL L^{-1} water
T5 = Spinomax	*Saccharopolyspora spinosa* + *Btk* broth	Russell IPM BD Ltd.	2 mL L^{-1} water
T6 = Fytoclean 40%	Potassium salt of fatty acid	Russell IPM BD Ltd.	5 mL L^{-1} water
T7 = Volium Flexi 300 SC	Chlorantraniliprole + Thiamethoxam	Syngenta BD	0.5 mL L^{-1} water

GLH/infested leaf) showed the lowest GLH abundance in each infested leaf while Biomax M (2.25 GLH/infested leaf) showed the highest abundance of GLH (Figure 4A). However, in the untreated control, GLH abundance was 2.83 GLH/infested leaf and in the chemical control of Voliam flexi, it was 0.22 GLH/infested leaf (Figure 4A). Compared to the untreated control, the maximum reduction of GLH abundance was achieved by Fizimite (54.34%) as well as Fytoclean (54.91%) while the lowest reduction was observed in Biomax M (20.37%) (Figure 4B). However, the chemical control Voliam flexi achieved a 92.16% reduction of GLH (Figure 4B).

Discussion

Leafhopper is a serious pest to brinjal that sucks cell sap and causes chlorosis, necrosis spots, and curling in the edges of the leaf resulting in droop off the leaves (Ghosh and Karmakar, 2021). It also interrupts photosynthesis by honeydew secretion and transmits pathogenic viruses to plants.

Among the treated bio-insecticides, a sodium lauryl ether sulfate preparation, Fizimite 20% was found effective against leafhoppers. It reduces 85.80% leafhopper population in the plant, a 77.84% reduction of leaf infestation, and a 54.34% reduction in the abundance of leafhoppers in the infested brinjal leaf. Sodium lauryl ether sulfate is an anionic highly soluble and non-volatile surfactant that helps to control chewing and sucking pests by physical means. It is effective against hoppers, aphids, mealybugs, whiteflies, spiders, scale, and psyllids (Lewis *et al.*, 2016). Another research by Gayathri and Geetha (2019) reported that applications of bio-pesticide, Buprofezin 25SC reduced 78.78% reduction of the leafhopper population in brinjal leaf. Another study by Ali *et al.* (2016) reported that neem extracts reduced 55.95 and 68.73% of Jassid/leafhopper populations in brinjal leaf after the first and second spray, respectively. Successive research showed that *Datura* extract reduced 63.11% and neem extract reduced 68.73% population of Jassid/leafhoppers (Ali *et al.*, 2017). Kunbhar *et al.* (2018), performed a study with some botanical pesticides (Neem, Tobacco, Trooh) and found that Neem was potent and reduced up to 56.09% of the Jassid population after seven days. The above discussion thus revealed that synthetic bio-pesticides are relatively more potent than botanicals or plant-origin insecticides. We found that the Synthetic bio-pesticide Buprofezin 25 SC provided a 78.78% leafhopper reduction, Fizimite

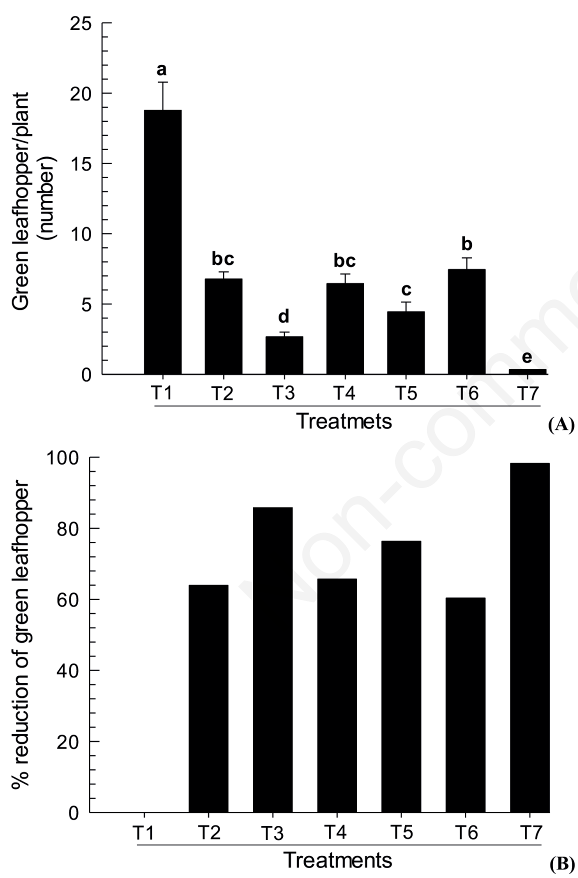

Figure 2. Effect of bio-insecticides on abundance of green leafhoppers. (A) Number of green leafhoppers per plant; (B) Green leafhopper number reduction over control. Bio-insecticides were applied at 10-day intervals on the whole plant. Control plants were sprayed with water. The number of green leafhoppers was recorded from the infested leaf. The mean value represents the average of six observations. The error bar above the mean value represents the standard deviation. Each treatment was replicated three times. Different letters above the error bar denote significant differences (P<0.05, least significant difference test) among the treatments.

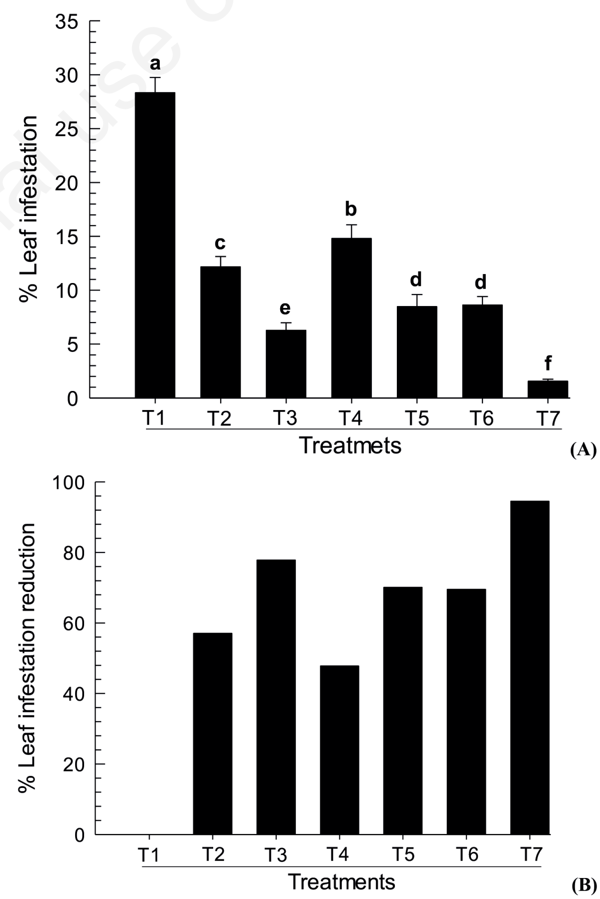

Figure 3. Effect of bio-insecticides on rate of leaf infestation. (A) Percent leaf infestation; (B) Reduction of leaf infestation by bio-insecticides over control. The number of infested and healthy leaves was recorded from the plants to work out the rate of infestation. The number represents the average of six observations. The error bar above the mean value represents the standard deviation. Each treatment was replicated three times. Different letters above the error bar denote significant differences (P<0.05, least significant difference test) among the treatments.

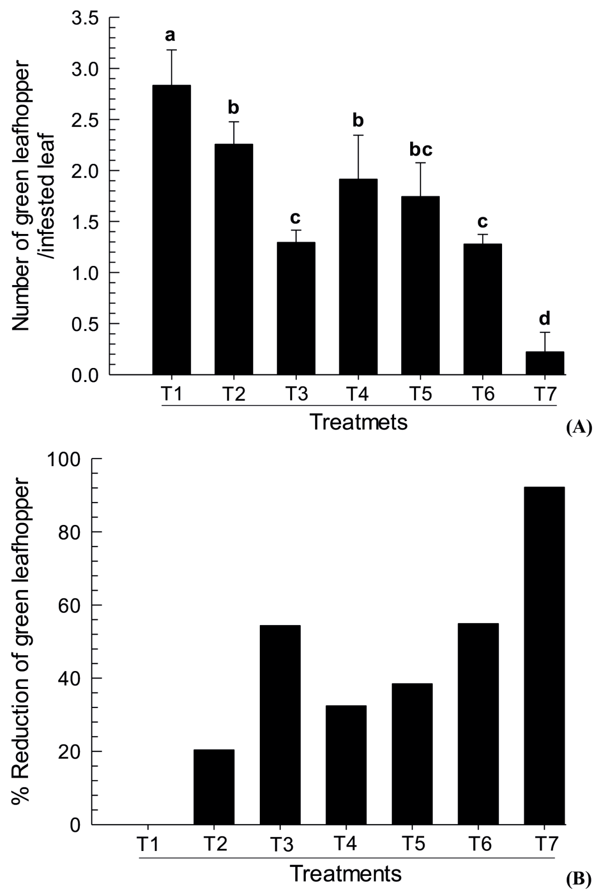

Figure 4. Effect of bio-insecticides on the presence of green leafhopper in the infested leaf. (A) Abundance of green leafhopper in the infested leaf (B) Reduction of green leafhopper abundance by bio-insecticides over control. The number of green leafhoppers in the infested leaf was counted. The number represents the average of six observations. The error bar above the mean value represents the standard deviation. Each treatment was replicated three times. Different letters above the error bar denote significant differences ($P<0.05$, least significant difference test) among the treatments.

20% provided a 85.80% leafhopper population reduction while among the botanical insecticides Neem extract provided a maximum 68.73% reduction of leafhopper population which is much less than Fizimite 20%. As leafhoppers are very active and relatively hard-bodied insects among the sucking insect pests, their control is not so easy. Considering all the issues, it can be concluded that Fizimite 20% is an effective bio-insecticide to control the leafhoppers in brinjal. However, further research on the impact of Fizimite 20% on the physiological process of leafhoppers might enhance the process.

Conclusions

The efficacy of bio-insecticides was evaluated against the leafhopper in the brinjal field during the winter season. A sodium lauryl ether sulfate preparation Fizimite 20% found effective against leafhoppers providing an 85.80% reduction of leafhopper number in the plant. It also provided a 77.84% reduction in leaf infestation by leafhoppers and a 54.34% reduction of leafhopper number in the infested leaf. These results followed the chemical insecticide, Voliam Flexi 300 SC. So, Fizimite 20% can be treated as a non-toxic alternative to chemical insecticides.

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