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Insecticidal Effects of Cattle Urine and Indigenous Plant Extracts Against Sugarcane Mealybugs

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Abstract: Laboratory investigation was carried out to evaluate the repellency and toxicity effect of fermented cattle urine, neem seed kernel, mahagoni seed and allamanda leaves extract @ 5, 10 and 15 percent concentration against mealybugs of sugarcane. All the extracts had repellent and toxic effect against mealybugs of sugarcane. The repellency effect of fermented cattle urine @ 15% was more (67.80%) than others. On the other hand, the mortality percentage of mealybugs was highest (68.66%) due to toxic effect of mahagoni seed extract @ 15% at 72 hours after treatment (HAT) than others in the same concentration and time. The repellency and mortality rate increased proportionately with the concentration and time. The LC_{50} values indicated that allamanda leaves extract (0.66%) at 24 HAT, fermented cattle urine (0.25%) at 48 HAT and mahagoni seed extract (0.07%) at 72 HAT were the most toxic. Comparing the three probit regression equations lines, the highest probit mortality was found with fermented cattle urine at 24, 48 and mahagoni seed extracts at 72 HAT.

Keywords: Animal and Plant Extracts, Insecticidal Effect, Mealybugs, Sugarcane

1. Introduction

Sugarcane is one of the important food-cum-industrial crops in Bangladesh. It is cultivated on an area of about 0.17 million hectares of lands that covers 2.05% of the total cultivable land and annual cane production is 4491 thousand metric tons [1]. It contributes 5% GDP of crop sector which covers 0.91% of national economy [2]. Despite a favorable land, soil type and agro-climatic condition, average per hectare yield of sugarcane in Bangladesh is only 41 tons and sugar recovery is 6.61-8.4% [2]. Among the reasons of low production and recovery of sugarcane, the insect pests constitute the major factor. Insect pest causes about 20% yield loss and 15% sugar loss every year in Bangladesh [3].Thirty species of mealybugs are known to attack sugarcane in different countries of the world [4]. They also reported 20% yield loss, 21.1 – 30% loss of sugar recovery and 16.2% loss of brix by mealybugs.

Insect pest problems and their management is a part of modern agriculture. Pest control measures, specially the use of chemical pesticides, however, have evoked a lot of controversy and debate vis-a-vis their deleterious effects on the environment and human health in recent times. Chemical control of insects has been used for a long time, but has serious drawback. The indiscriminate use of chemical pesticides has resulted in adverse effects like resistance [5, 6], ecological imbalance, health hazards and resurgence of secondary pest. Through the import of 3 metric tons of insecticides in 1956, Bangladesh entered into the era of using synthetic chemical pesticides for pest control. In Bangladesh during 1996-97 to 2007-08, pesticide consumption increased 328.4% and per hectare pesticide use increased 598.8% [7].

To overcome these problems identification of safe molecules with better insecticidal properties having lower

mammalian toxicity, safe to natural enemies etc., which fits well in the IPM concept are needed at present. Scientists are diverting their attention worldwide to the animal and plant products due to their biodegradability and safety to natural enemies [8]. Many plant and natural products have been reported to possess insecticidal activity, repellency to pests, antifeedancy, insect growth regulation etc. It has been reported that around 866 plant species have activity against insect species [9]. Considerable work on the insecticidal activity of cattle urine and indigenous plant extracts had been done in many countries in the world. But only a few attempts have been made to evaluate natural products like cattle urine and plant extracts i.e., neem, mahagoni and allamanda against insect pests in Bangladesh [10]. Therefore, the present study was undertaken to find out the effectiveness of cattle urine and plant extracts against mealybugs infesting sugarcane.

2. Materials and Methods

2.1. Methodology

The present study was conducted in the laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) during cropping season 2013-14.

2.2. Preparation of Cattle Urine and Plant Extracts

Cattle urine was collected from different cattle of Gazipur, Bangladesh. After collection, it was kept underground in an earthen pot for 14 days for fermentation. Then 50, 100 and 150 ml of fermented cattle urine was diluted with 1 liter of water to make up the concentration to 5, 10 and 15%. Fifty, 100 and 150 grams of neem and mahagoni seeds was shade dried, crushed and then soaked overnight in 1 litre of water to get 5, 10 and 15% concentration. Later, the mixture was squeezed through the muslin cloth. To get 5, 10 and 15% concentration of allamanda leaves extract 50, 100 and 150 grams of pieces allamanda leaves were blended with 1 litre of water.

2.3. Insect Bioassays

Insect bioassays were done to determine the repellency, relative toxicity, mortality percentage and probit regression line.

2.4. Repellency Test

Repellency test was conducted following the method of Talukder & Howse (1994 a, b) [11, 12] with slight modification. The freshly collected sugarcane leaves (10 cm) were divided into two portions: 5.0 cm treated and 5.0 cm untreated. The two portions were separated by a hard paper of 0.5 cm height. Five pair of insects was released in the petridish (15 cm diam.). Four treatments along with a control and five replications were made for each treatment. Then the number of insects in each portion was counted at hourly interval up to the 4^{th} hour. The data were expressed as percentage repulsion (PR) by the following formula:

$$PR\ (\%) = (Nc - 50) \times 2$$

Where, Nc = The percentage of insects present in the control half. Positive (+) values expressed repellency, and Negative (-) values attractancy. Data (PR %) was analyzed using ANOVA after arcsin√percentage transformation. The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V: class 0 (PR=0.01-0.1%), class I (PR=0.1-20%), class II (PR=20.1-40%), class III (PR=40.1-60%), class IV (PR=60.1-80%) and class V (PR=80.1-100%) [13].

2.5. Toxicity Test

The toxicity test was conducted according to the method described by Talukder and Howse (1995) [14] with slight modification. The freshly collected sugarcane leaves (10 cm) were dipped in three concentrations of fermented cattle urine and indigenous plant extracts for 10 minutes before use. Then dipped leaf was placed in a 15 cm diameter petridish and covered with lid. Four treatments along with a control and five replications were made for each treatment. Insect mortalities were recorded at 24, 48, and 72 hours after treatment (an insect was considered dead if it did not move the antenna or the legs when touched twice with tweezers).

2.6. Statistical Analysis

The observed values obtained during the experiments were compared by Tukey's studentised range test. For mortality test, original data were corrected by Abbott's (1925) [15] formula and then analyzed using ANOVA. The median lethal concentration (LC_{50}) was calculated using probit analyses [16] with a $\log_{10}$ transformation of concentrations of extracts by Statistical Analysis Software (SAS) 9.1 Windows version. LC_{50} and toxicity relationship reverse i.e., the lower the amount, the more toxic the material.

3. Results and Discussion

Fermented cattle urine, neem seed kernel extracts, mahagoni seed extracts and allamanda leaves extracts showed repellent effect against mealybugs of sugarcane. But 15% concentration showed better performance than other concentrations in all cases. The repellency percentage and scale for the treatments tested are shown in Table 1.The highest repellency (81.38%) was observed @ 15% fermented cattle urine at 1 HAT followed by mahagoni seed extract (47.70%) at same concentration. The repellency rate increased proportionately with the concentration and time. At 4 HAT, the highest repellency (87.05%) was recorded in fermented cattle urine @ 15% followed by mahagoni seed extract (75.70%) at same concentration. The highest mean repellency (67.80%) was observed @ 15% fermented cattle urine which show the repellency scale IV, followed by mahagoni seed extracts (52.80%) @ 15%, fermented cattle

urine (48.79%) @ 10% and neem seed extracts (41.80%) @ 15% and repellency scale was the same (III).

Table 1. *Repellency rate of different concentrations of fermented cattle urine and indigenous plant extracts against mealybugs of sugarcane at BSMRAU laboratory, Gazipur, Bangladesh, 2013.*

Name of the extracts	Conc (%)	Average repellency rate (%)				Mean of repellency rate (%)	Repellency scale
		1 HAT	2 HAT	3 HAT	4 HAT		
FCU	5	29.15 bc	12.08 bc	16.28 b	65.38 abc	30.72	II
	10	60.75 ab	24.51 abc	28.54 ab	81.38 a	48.79	III
	15	81.38 a	46.23 a	56.54 a	87.05 a	67.80	IV
NSKE	5	20.31 c	20.31 bc	16.28 b	37.39 cd	23.57	II
	10	24.51 bc	16.28 abc	16.28 b	46.23 bcd	25.82	II
	15	46.23 abc	28.54 abc	37.39 ab	55.07 abc	41.80	III
MSE	5	12.08 c	12.08 bc	32.75 ab	37.39 cd	23.57	II
	10	29.15 bc	28.54 abc	28.54 ab	55.07 abc	35.32	II
	15	47.70 abc	41.59 ab	46.23 ab	75.70 ab	52.80	III
ALE	5	20.31 c	12.08 bc	16.28 b	20.31 d	17.24	I
	10	12.08 c	8.05 c	12.08 b	37.39 cd	17.40	I
	15	16.11 c	20.31 abc	24.51 ab	37.39 cd	24.58	II
SE		11.12	4.48	10.37	10.03		

Legend: HAT: Hours after treatment, FCU: Fermented cattle urine, NSKE: Neem seed kernel extracts, MSE: Mahagoni seed extracts and ALE: Allamanda leaves extracts.
Means in a column followed by different letters (a, b, c, d) are significantly different at α=0.05 by Tukey's studentised range (HSD) test.

The lowest mean repellency was observed (17.24%) @ 5% allamanda leaves extract, followed by 10% allamanda leaves extract (17.40%). The repellency effect of all the treatments at different concentrations was statistically different. Uddin [17] observed that mahagoni oil performed the highest repellency after 24 HAT followed by neem oil and eucalyptus oil. This result was at par with the present results on repellent effect of neem and mahagoni seed extracts. Parvin et. al. [18] found the highest repellency (93.30%) of *T. castaneum* occurred at the highest concentration (5.0%), while the lowest (0.0%) repellency occurred at 0.5% suspension after 1 day of treatment by mahagoni extracts.

Mortality percentage indicated that mahagoni seed extracts at highest concentration (15%) possessed the highest toxic effect on mealybugs (68.66%) at 72 HAT, followed by neem seed kernel extracts (58.22%) and fermented cattle urine (56.22%) in the same concentration and time. Allamanda leaves extracts @ 5% showed the lowest mortality (29.55%) at 72 HAT (Table 2). The order of toxicity was mahagoni>neem>cattle urine>allamanda which might be due to the chemical composition of them. The mortality rate increased proportionately with the concentration and time. Parvin et. al. [18] found the highest mortality against adults (86.71%), larvae (88.32%) and pupae (85.00%) occurred at 5% suspension after 8 days of application by mahagoni extracts.

Table 2. *Mean mortality percentages of sugarcane mealybugs with different concentrations of fermented cattle urine and indigenous plant extracts, BSMRAU, Gazipur, 2013.*

Name of the extracts	Conc (%)	No. of insects treated	Average insect mortality (%) at		
			24 HAT	48 HAT	72 HAT
FCU	5	50	26.00 bc	36.00 bc	54.88 a
	10	50	30.00 bc	40.00 bc	48.44 a
	15	50	34.00 b	46.00 bc	56.22 a
	Control	50	0.00 a	0.00 a	2.00 a
NSKE	5	50	20.00 cd	30.44 bc	39.33 a
	10	50	26.00 bc	34.66 bc	43.55 a
	15	50	30.00 bc	36.66 bc	58.22 a
	Control	50	0.00 a	2.00 ab	4.00 a
MSE	5	50	24.00 bc	32.22 bc	45.55 a
	10	50	28.00 bc	36.66 bc	51.99 a
	15	50	36.00 b	44.88 bc	68.66 a
	Control	50	0.00 a	2.00 ab	4.00 a
ALE	5	50	14.00 d	22.66 c	29.55 a
	10	50	20.00 cd	22.44 c	38.22 a
	15	50	26.00 bc	31.11 bc	46.88 a
	Control	50	0.00 a	4.00 abc	6.00 a
SE			0.028	0.093	0.117

Legend: HAT: Hours after treatment, FCU: Fermented cattle urine, NSKE: Neem seed kernel extracts, MSE: Mahagoni seed extracts and ALE: Allamanda leaves extracts.
Ten insects were treated as a replication.
Original data corrected by Abbott's formula and then transformed into $\log_{10}$ values before ANOVA and Tukey's studentised range (HSD) test.
Values followed by the same letter within a column are not significantly different at the 0.05 level (HSD test).

3.1. Probit Analysis of Toxicity

The results of probit analysis for the estimation of LC_{50} values and slopes of regression lines of 24, 48 and 72 HAT for the mortality of mealybugs of sugarcane are presented in Table 3. LC_{50} and toxicity relationship reverse i.e., the lower the amount, the more toxic the material. The LC_{50} values of fermented cattle urine (1.11%), neem seed kernel extract (0.82%), mahagoni seed extract (0.77%) and allamanda leaves extract (0.66%) at 24 HAT indicated that allamanda leaves extract was the most toxic. At 48 HAT, comparison of LC_{50} values showed that fermented cattle urine found the highest toxic effect (0.25%), whereas neem seed kernel extract demonstrated the lowest (1.47%) which might be due to the chemical constituents. At 72 HAT, it was found that mahagoni seed extract (0.07%) maintained its highest toxicity. Slope provides the rate of changes in mortality with dosages, a measure of homogeneity.

Table 3. *Relative toxicity (by probit analysis) of fermented cattle urine and three plant extracts against mealybugs of sugarcane at BSMRAU, Gazipur. 2013.*

Name of the extracts	No of insects	LC_{50} (%)	Slope
FCU			
24 HAT	200	1.11	0.83
48 HAT	200	0.25	0.84
72 HAT	200	0.21	0.07
NSKE			
24 HAT	200	0.82	1.21
48 HAT	200	1.47	0.57
72 HAT	200	0.12	1.43
MSE			
24 HAT	200	0.77	1.04
48 HAT	200	0.29	1.02
72 HAT	200	0.07	2.15
ALE			
24 HAT	200	0.66	1.75
48 HAT	200	0.60	1.27
72 HAT	200	0.24	1.50

Legend: LC_{50}: Median lethal concentration, HAT: Hours after treatment, FCU: Fermented cattle urine, NSKE: Neem seed kernel extracts, MSE: Mahagoni seed extracts and ALE: Allamanda leaves extracts.
No. of insects were based on four concentrations (including control) and five replicates of 10 insects each.
All data were transformed by log transformation before analyses.

3.2. Probit Regression Lines

The probit regression lines for the effects of fermented cattle urine, neem seed kernel extract, mahagoni seed extract and allamanda leaves extract on mealybugs of sugarcane are presented in Figure 1, 2, 3 and 4. The insect mortality rate showed positive correlation with doses and time in all cases. The probit regression lines for the effects of four different extracts showed a clear linear relationship between probit mortality and their log doses.

The probit regression lines became steeper as doses increased, because the insects were treated with more toxic one for the same period at higher doses. The calculated probit regression equation lines for fermented cattle urine, extracts of neem seed kernel, mahagoni seed and allamanda leaves at 24 HAT were y = 0.495x + 3.957, y = 0.702x + 3.608, y = 0.621x + 3.792 and y = 0.976x + 3.145, respectively.

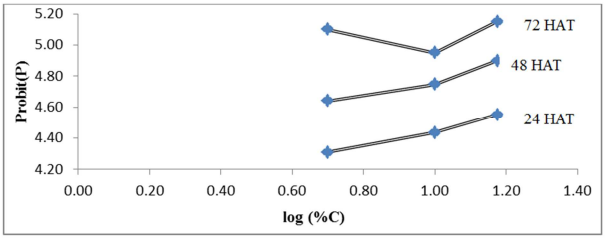

Figure 1. *Relationship between probit mortality and log of doses of fermented cattle urine on mealybugs of sugarcane at 24, 48 and 72 HAT.*

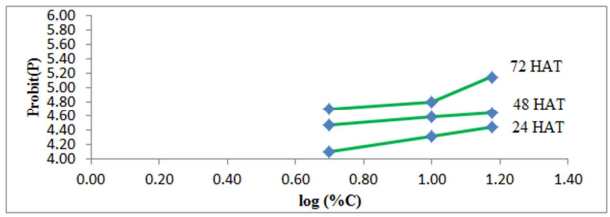

Figure 2. *Relationship between probit mortality and log of doses of neem seed kernel extract on mealybugs of sugarcane at 24, 48 and 72 HAT.*

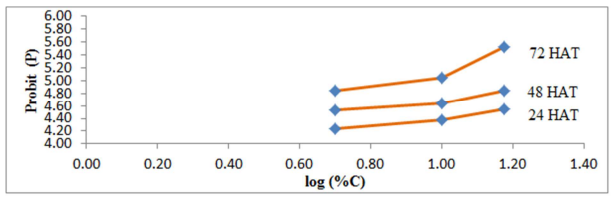

Figure 3. *Relationship between probit mortality and log of doses of mahagoni seed extract on mealybugs of sugarcane at 24, 48 and 72 HAT.*

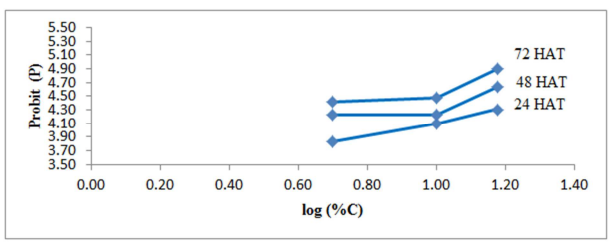

Figure 4. *Relationship between probit mortality and log of doses of allamanda leaves extract on mealybugs of sugarcane at 24, 48 and 72 HAT.*

The calculated probit regression equations for the lines at 48 HAT were: y = 0.520x + 4.264 for fermented cattle urine, y = 0.350x + 4.232 for neem seed kernel extract, y = 0.631x + 4.069 for mahagoni seed extract and y = 0.759x + 3.635 for allamanda leaves extract. At 72 HAT, the regression equation lines for different extracts were: y = 0.040x + 5.028, y = 0.890x + 4.027, y = 1.335x + 3.861 and y = 0.923x + 3.712 for urine, neem, mahagoni and allamanda respectively. Comparing the three probit regression equations lines, the highest probit mortality was found with fermented cattle urine at 24, 48 and mahagoni seed extracts at 72 HAT.

4. Conclusion

Out of one animal product and three plants screened for efficacy as eco-friendly insecticide, cattle urine and mahagoni seed extracts were the most effective. The repellency effect against mealybugs of fermented cattle urine sprayed @ 15% was more than the plant extracts. On the other hand, the insect mortality percentage of mealybugs was highest due to toxic effect of mahagoni seed extract @ 15% at 72 hours after treatment (HAT) than others. The LC_{50} values of allamanda leaves extracts were 0.66% at 24 HAT which was the most toxic. This was followed by fermented cattle urine (0.25%) at 48 HAT and mahagoni seed extract (0.07%) at 72 HAT.

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