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Assessment of Toxic and Repellent Effect of Natural Bio Pesticides on Rice Weevil (Sitophilus Oryzae L.)

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Abstract: *With a wider view to maintaining sound ecology and health, the study was designed to assess the toxic and repellent effect of natural bio pesticides named Neem (Azadirachta indica), Mahogoni (Swietenia mahagoni) and their mixtures at 0%, 1%, 2% and 3% concentrations on rice weevil, Sitophilus oryzae L using factorial CRD with 2 factors. The results showed that the highest toxic and repellent effects were observed in Neem followed by mixture and Mahogoni, while insect mortality percentage was found to be directly proportional not only to the time after treatment but also to the level of concentration of the botanicals, and the repellent effect increases proportionally to the level of concentration of botanicals. Moreover, the results showed that 3% concentration of the botanicals had the highest toxicity and repellency against rice weevil followed by 2% and the lowest was found in control (0%) preceded by 1% concentration.*
Key words: botanicals, rice weevil, repellency and toxicity

I. Introduction

In Bangladesh, rice is the staple food for all people. That is why growers try to store unhusked rice grain in different vessel for year round usage. But it is fact that in storage condition a great loss or damage occurs due to stored grain pests, is a serious threat to our national economy. In every year 5-8 percent of the food grains, seeds and different stored products are lost due to insect pests which would be up to 10 percent. Rice weevil Sitophilus oryzae L. (Coleoptera; curculionidae) is a major storage pest of economic importance over the world and decline the food value in stored cereal grains. Around 15 percent rice on storage is lost due to stored pests of which rice weevil, S. oryzae L. is one of the most significant damaging pests [1].

The pest not only damages the grain but also deteriorates the weight and quality of it. It causes damage similar to that of the maize weevil and the granary weevil. In storage condition rice weevils can cause serious damage to grain directly through consumption of the grain or indirectly by producing 'Hot spot' causing loss of moisture and thereby making grain more suitable for their consumption. Rice weevils not only damage the stored rice (both husked and unhusked) but also maize, wheat and sorghum particularly in monsoon. The damage in storage is more crucial than in the field [2].

In order to preserve the grain it is necessary to make a constant war with stored grain pests which inflict considerable damage to the cereal. Many preventive measures have been reported to minimize the loss due to rice weevil. Among these, chemical insecticides can be used for effective control but they have some serious drawbacks such as undesirable side effects, toxic residues, costly application and finally health hazards to consumer (Human and Animal). It also pollutes air, water, soil that means whole environment become in threat. Besides, continuous use of chemical insecticides develops cross and multiple resistant strains in many important insect species [3].

In this circumstance, a worldwide attention in the development of alternative strategies of chemicals that involves the search for new types of botanicals insecticides and use of age old traditional pest control agents are noticeable because plant-derived pesticides can be transferred into practical applications in natural crop protection, which can help the small-scale farmers [4]. The use of natural and easily biodegradable crop protection inputs can be a useful component of an IPM strategy since the compound is known for its low toxicity against beneficial insects. Moreover, botanical insecticides have no or less side effects and toxic residues which are non-hazardous to consumers and other vertebrates, and it can easily be produced by farmers that are less expensive and safe and amenable to apply [5]. But the scientific research work in this area is so sketchy in our country that the study was undertaken to evaluate the repellency and toxicity of natural bio pesticides viz. Neem and Mahogoni seed extracts along with their mixture on rice weevil.

II. Methodology

The study was conducted in laboratory condition maintaining temperature and humidity of 25 ± 3^0C and 73±5% respectively in order to assemble valid and reliable data for achieving a meaningful result and conclusion.

2.1. Neem Seed Extract

All most all parts of Neem tree (Azadirachta indica: Meliaceae) especially its seeds kernel and leaves are very important culturally and economically because of insecticidal, antiviral, bactericidal, antifeedant, antifungal, larvicidal, nematicidal and other biological activities of its derivatives. The active ingredient of Neem is Azadirachtin which is isolated from the seed and it is very useful for pest and disease control [6].

2.2. Mahogoni Seed Extract

Mehagoni (Swietenia mahagoni: Meliaceae) has significant pesticidal usage to control the insect pests in agriculture. Now a days, the leaves and seed extracts of Mahogoni are maximally used as bio-control agent in the management of insect pests both in field and storage [6].

2.3. Mixture

Mixture was prepared manually in Laboratory by mixing equal quantity of both Neem and Mahogoni seed extracts in together. It plays significant role in controlling pests both in field and storage condition.

2.3. Collection and Rearing of Test Insects

Rice weevils were collected from stock culture of the Entomology Laboratory, Agrotechnology Discipline, Khulna University, Khulna. Rice weevils were reared in a big plastic jars (radius 34 cm and height 22 cm) in which sterile rice was given in jar for insect feeding and the jar was kept in the Laboratory maintaining temperature and humidity of 25 ± 3^0C and 73±5% respectively for two month to multiply insect number. To ensure continuous supply of adequate adult, the rearing procedure was repeated with different batches of insects.

2.4. Treatment and Design

The experiments were laid out in factorial CRD with two factors (botanicals x concentration) and three replicas. Factor A involved two locally available botanicals namely Neem, Mahogoni seed extracts and their mixture, and factor B comprised different concentrations viz. 1.0%, 2.0%, 3.0% and an untreated control (0%) of the botanicals. Thus, the number of treatment combinations was shown as 3 botanicals x 4 concentrations.

2.5. Toxicity Test

For toxicity test, five pair of rice weevils was taken to the petridish containing 10g rice treated with different concentrations of two botanicals' seed extracts and their mixture. Same number of insects was kept in untreated medium as control. The adult mortality was recorded from each replication and the data was corrected and converted into percentage from the original data by the Abbott's following formula [7].

$$\text{Corrected mortality} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

2.6. Repellency Test

In repellency test, petridishes were divided into two parts: treated and untreated. With the help of pipette, plant seed extracts were applied to one half of the grains. After air drying of treated half, ten insects were released at the centre of each petridish. Then the numbers of insects in each portion were counted at hourly interval up to the third hour. The data were expressed to 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) were analyzed using analysis of variance (ANOVA). Then the average values were categorized according to the following class [8].

Class	Repellency rate (%)
0	>0.01 to 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

2.7. Statistical Analysis

The recorded data were analyzed statistically for ANOVA with the help of computer aided package program MSTAT-C and the mean differences were adjudged by Duncan's New Multiple Range Test [9].

III. Result and Discussion

3.1. Toxicity Test

The toxic effects of natural bio pesticides (Neem, Mahogoni and the mixture of Neem and Mahogoni), their different concentrations (0% (control), 1%, 2%, and 3%) and both botanicals and their different concentrations at every 24 hours after treatment on rice weevil until death was evaluated in this experiment and presented in Table 1 and Table2, and Figure 1 and Figure 2.

3.1.1. Toxic effect of botanicals on the mortality of the test insects (S. oryzae)

The toxic effect of two botanicals namely Neem and Mahogoni and their mixture on the rice weevil S. oryzae at 24, 48, 72, 96 and 120 hours after treatment (HAT) was found statistically insignificant (Fig. 1) along with average daily mortality (Fig. 2). But numerically, the results indicated that all of the botanicals possessed the lowest mortality at 24 HAT and the highest at 120 HAT. At 24 HAT, among three botanicals, Mahogoni claimed the lowest mortality (13.33%) preceded by mixture (15.83%) and the highest mortality (17.50%) was found against Neem. At 120 HAT, mixture (Neem+ Mahogoni) caused the highest mortality (64.32%) followed by Neem (63.98%) due to high toxicity and the lowest mortality (57.38%) against Mahogoni due to low toxic effect (Fig. 1).

In addition, the highest average daily mortality (12.86%) was observed in case of the mixture of Neem and Mahogoni and the lowest average daily mortality (11.48%) was observed in case of Mahogoni (Fig. 2).

So, the order of toxicity for three botanicals on rice weevil, S. oryzae L. was shown as mixture (Neem+Mahogoni)>Neem>Mahogoni.

But it was noticed that upto four consecutive days, Neem claimed the highest mortality percentage and at 5^{th} day last (120 HAT), mixture of Neem and Mahogoni possessed the highest mortality on the test insect among three botanicals. On the other hand, Mahogoni was found the lowest to kill the insects for four days along with average daily mortality except 96 HAT among Neem, Mahogoni and their mixture. Here, it was worth mentioning that the mortality percentage of the test insects increased with the increase of time until their death. It is reported that Neem seed extracts caused 74% mortality of rice green leaf hopper (Nephotettix cinccticeps) in vivo and in vitro condition at 72 hours after treatment (HAT) [10] which was much higher than our result (46.48%) obtained against rice weevil at same time after treatment. It might be due to distinct difference of test insects, laboratory environment along with variation in doses of botanicals.

In all, the disparity of mortality percentage of the test insects might be owing to variation in degree of presence and functioning of toxic ingredients along with distinct difference in nature and structure of the botanicals used in the experiment.

3.1.2. Toxic effect of different concentrations of the botanicals on the mortality of the test insects (S. oryzae)

The toxic effect of different concentrations of botanicals on the rice weevil, S. oryzae at 24, 48, 72, 96 and 120 hours after treatment (HAT) varied significantly from one another (Table 1). It was quite evident that all of the concentrations possessed the lowest mortality at 24 HAT and the highest at 120 HAT. At 24 HAT, the lowest mortality (0%) was measured in control (0%) i.e. untreated seed and the highest mortality (28.89%) produced in 3% concentration. At 120 HAT, 3% concentration caused the highest mortality (89.15%) followed by 2% concentration (76.92%) due to its high concentration and toxicity, and the lowest mortality (16.67%) was measured in control (0%) i.e. untreated seed.

Moreover, the highest average daily mortality (17.83%) was observed in 3% concentration and the lowest average daily mortality (3.33%) was observed in case of control (0%). But in case of treated seed, it was recorded that the lowest and highest mortality was always found in 1% and 3% concentration respectively and till five consecutive days after treatment along with average daily mortality.

From the results, it was distinct that mortality of insect increased with the increase of concentration level of botanicals which was supported by [11] where they observed same phenomenon on Oryctes rhinoceros by the action of Neem oil concentrations. So, the order of toxicity of three concentrations and control on rice weevil, S. oryzae L. could be ranked as 3%>2%>1%>control (0%).

However, the dissimilar mortality percentage of test insects caused by three botanicals might be due to significant variation among the concentrations of the botanicals along with disparate composition, presence and functioning of noxious ingredients present in the different concentrations of botanicals used in the study.

3.1.3. Combined effect of both botanicals and their concentrations on the mortality of test insects (S. oryzae)

The toxic effect of both botanicals (Neem, Mahogoni and the mixture of Neem and Mahogoni) and their different concentrations (1%, 2%, 3%) with control (0%) on the rice weevil S. oryzae at 24, 48, 72, 96 and 120 hours after treatment (HAT) were not found statistically significant (Table 2). But numerically, the results depicted that at 24 HAT, the lowest mortality (0%) was recorded in control (0%) of all botanicals preceded by 1% of both Neem and Mahogoni (13.33%) and Neem caused the highest mortality (33.33%) with its 3% concentration followed by 2% concentration (23.33%). At 120 HAT, 3% concentration of Neem showed the highest mortality (96.30%) followed by its 2% concentration (80.56%) due to its high concentration and toxicity, and the lowest mortality (13.33 %) was measured in control (0%) of both Neem and Mahogoni owing to no toxic effect.

Thus, the order of toxicity of both botanicals and their concentrations on rice weevil, S. oryzae L. were presented as

Neem(3%)>mixture(3%)>Neem(2%)>Mahogoni(3%)>mixture(2%)>Mahogoni(2%)>
Neem(1%)>mixture(1%)> Mahogoni(1%)>control.

The variation in mortality percentage of the test insects (rice weevil) might be due to difference in the concentrations (0%, 1%, 2% and 3%) of botanicals having unequal functioning and proportion of toxic ingredients.

3.2. Repellency Test

The repellent effect of natural bio pesticides (Neem, Mahogoni and the mixture of Neem and Mahogoni), their different concentrations (1%, 2% and 3%) and both botanicals and their concentrations at different hours after treatment on rice weevil were evaluated in this experiment and presented in Table 3 and 4, and Figure 3 and 4.

3.2.1. Repellent effect of botanicals on the test insects (S. oryzae)

The repellency rate of two botanicals and their mixture at different hours after treatment showed both statistical significant and insignificant effect on rice weevil (Table 3). The table specified that botanicals attained the highest repellent effect at 1 HAT when Neem repelled the most insects (66.67%) and the least insects (48.89%) were repelled by mixture. On contrary, the lowest repellent rate was found at 3 HAT when the highest (48.89%) and the lowest (40%) repellency were against mixture and Mahogoni respectively.

Again, the highest average repellency per hour caused by Neem (58.52%) followed by mixture, Neem + Mahogoni (51.85%) and the lowest by Mahogoni (51.11%). It was noteworthy that at 3 HAT mixtures showed the highest repellent effect among three botanicals even though Neem claimed the highest repellency till 2 HAT. However, all the botanicals used in the study attained the same repellency class (III) according to percent repellency rate.

It was reported that castor oil [12] showed the highest repellent effect (100%) on C. maculates infested stored green gram followed by Neem leaf powder (91.66%) which was much higher than our documented result. It might be due to distinct difference of test insects, laboratory environment along with variation in formulation of botanicals. In this study, the unequal repellent percentage on the test insects (rice weevil) might be due to variation in the degree of odor, existence of repelling compounds and activity of the botanicals along with their difference in nature and structure.

3.2.2. Repellent effect of different concentrations of botanicals on the test insects (S. oryzae)

The different concentrations (1%, 2% and 3%) of the botanicals at different hours after treatment caused statistical significant repellent effect on rice weevil (Table 4). Among 3 concentrations and 3 times i.e. 1, 2 and 3 HAT, the highest number of insects (71.11%) was repelled by 3% concentration at both 1 and 2 HAT, and the lowest (26.67%) was repelled by 1% concentration at 3 HAT.

Again, the highest average repellent intensity per hour (66.67%) caused by high concentration (3%) followed by medium concentration (2%) repelling (58.52%) and the lowest (36.30%) caused by low concentration (1%).The repellent effect of different concentrations of all botanicals increased with the increase

of concentrations which was supported by [13] where they observed alike repellent trend on Corcyra cephalonica by the action of different concentrations (0.1%, 0.3% and 0.5%) of various plants' products namely clove, cedar wood, citronnela and eucalyptus oil .

According to repellent intensity, the order and repellency class of various concentrations of botanicals could be expressed as 3%> 2%> 1% and IV>III>II respectively. However, the disparity of repellent effect among three concentrations of botanicals on the test insects (rice weevil) might be owing to difference in the degree of concentrations which regulated their repellent strength as well as persistence of odorous compounds present in the concentrations of the botanicals.

3.2.3. Repellent effect of both botanicals and their concentrations on the test insects (S. oryzae)

The repellent effect of both botanicals (Neem, Mahogoni and the mixture of Neem and Mahogoni) and their different concentrations (1%, 2%, and 3%) on rice weevil at different hours after treatment was not statistically significant (Fig. 3) along with average per hour repellency (Fig. 4). But numerically, the figure revealed that among three botanicals along with its three concentrations, the highest percent of insects (86.67%) were repelled by 3% concentration of Neem at 2 HAT when the lowest (20.00%) was against 1% of mixture. On the other hand, the lowest repellency was recorded in 1% concentration of all botanicals at 3 HAT when the highest (66.67%) was in 3% of mixture (Fig. 3). In addition, the highest average repellent intensity (75.56%) per hour caused by Neem with its maximum concentration (3%) and the lowest (31.11%) was caused by mixture of Neem and Mahogoni with minimum concentration of 1% (Fig. 4).Thus, the result explored that repellent effect of botanicals was increased with the increase of concentrations and vice versa. The dissimilarity of repellent effect of botanicals and their concentrations on the test insects (rice weevil) could be owing to the fact that there were distinct difference in the fluctuation of concentrations (1%, 2% and 3%) in their quantity along with disparate composition, existence and action of repelling ingredients in the botanicals.

IV. Conclusion

This study addressed a set of eco-friendly management practices to evaluate the toxic and repellent effect of natural bio pesticides named Neem, Mahogoni and their mixture on rice weevil (S. oryzae) to protect unhusked rice seed damage. Out of three botanicals along with their four concentrations viz. 0, 1, 2, and 3%, Neem and mixture with their 3% concentration were proved the most effective against rice weevil in respect of its mortality, repellency. So, the order of both botanicals and concentration could be ranked as Neem ≥ mixture> Mahogoni and 3%> 2%> 1%> 0% respectively. The study to improve the effectiveness of botanical derivatives as alternatives of synthetic insecticides will benefit our agriculture sectors, as these botanicals are not only of low cost but also have less environmental impact in comparison to insecticidal hazard. Therefore, it can be concluded that this study established the fact that the locally available natural bio pesticides can be exploited as a very useful ecofriendly weapon to protect unhusked unboiled rice from the severe infestation of rice weevil in storage condition.

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Table 1. Effect of different concentrations of the botanicals on the mortality of the test insects (S. oryzae)

Conc. (%)	Mortality of the Test Insect (%)					
	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	Mortality per day
1	14.44 b	30.86 a	48.64 b	57.72 b	64.84 b	12.97 b
2	18.89 ab	35.31 a	54.57 ab	66.51 ab	76.92 ab	15.38 ab
3	28.89 a	45.81 a	67.65 a	79.48 a	89.15 a	17.83 a
0	0.00 c	3.33 b	6.67 c	12.22 c	16.67 c	3.33 c
LS	* *	* *	* *	* *	* *	* *
Mean	15.53	28.83	44.38	53.98	61.89	12.37
SE±	5.20	7.84	11.42	12.66	13.74	2.75

HAT = Hour after treatment
LS = Level of significance
* * = Significant at 1% level
SE = Standard error
Conc. = Concentration
Within the table different letter(s) in same column indicate that they are significantly different by DMRT.

Table 2. Effect of both botanicals and their concentrations on the mortality of the test insects (S. oryzae)

Name of the Botanicals	Mortality of the Test Insect (%)						
	Conc. (%)	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	Mortality per day
Neem	1	13.33	30.74	51.85	59.26	65.74	13.15
	2	23.33	41.11	58.52	70.37	80.56	16.11
	3	33.33	44.81	72.22	85.19	96.30	19.26
	0	0.00	3.33	3.33	10.00	13.33	2.67
Mahogoni	1	13.33	30.74	45.93	56.02	63.89	12.78
	2	16.67	30.74	53.33	68.06	72.22	14.44
	3	23.33	44.44	64.07	76.39	80.09	16.02
	0	0.00	3.33	6.67	13.33	13.33	2.67
Mixture	1	16.67	31.11	48.15	57.87	64.88	12.98
	2	16.67	34.07	51.85	61.11	77.98	15.60
	3	30.00	48.15	66.67	76.85	91.07	18.21
	0	0.00	3.33	10.00	13.33	23.33	4.67
LS		NS	NS	NS	NS	NS	NS
Mean		15.55	28.83	44.38	53.98	61.89	12.38
SE±		3.10	4.58	6.64	7.36	8.04	1.61

HAT = Hour after treatment
LS = Level of significance
NS = Non significance
SE = Standard error
Conc. = Concentration

Table 3. Repellent effect of botanicals on the test insects (S. oryzae)

Name of The Botanicals	Repellency Rate			Repellency Per Hour	Repellency Class
	1 HAT	2 HAT	3 HAT		
Neem	66.67a	64.44	44.44	58.52	III
Mahogoni	55.56ab	57.78	40.00	51.11	III
Mixture	48.89b	57.78	48.89	51.85	III
LS	*	NS	NS	*	
Mean	57.04	60	44.44	53.83	
SE±	5.19	2.22	2.57	2.36	

HAT = Hour after treatment
LS = Level of significance
* = Significant at 5% level
NS = Non significance
SE = Standard error
Within the table different letter(s) in same column indicate that they are significantly different by DMRT.

Table 4. Repellent effect of different concentrations of botanicals on the test insect (S. oryzae)

Conc. (%)	Repellency Rate			Repellency Per Hour	Repellency Class
	1 HAT	2 HAT	3 HAT		
1	33.33 b	48.89 b	26.67 b	36.30 c	II
2	66.67 a	60.00 ab	48.89 a	58.52 b	III
3	71.11 a	71.11 a	57.78 a	66.67 a	IV
LS	* *	*	* *	* *	
Mean	57.04	60	44.44	53.83	
SE±	11.92	6.41	9.25	9.08	

HAT = Hour after treatment
LS = Level of significance
* * = Significant at 1% level
* = Significant at 5% level
SE = Standard error
Conc. = Concentration
Within table different letter(s) in same column indicate that they are significantly different by DMRT.

Figure 1. Effect of botanicals on the mortality of the test insects (S. oryzae)

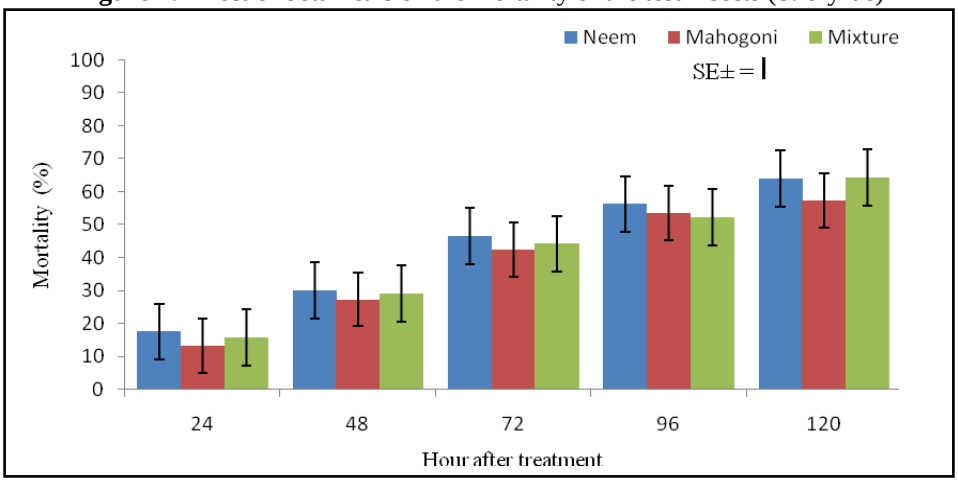


Figure 2. Effect of botanicals on the average daily mortality of the test insects (S. oryzae)

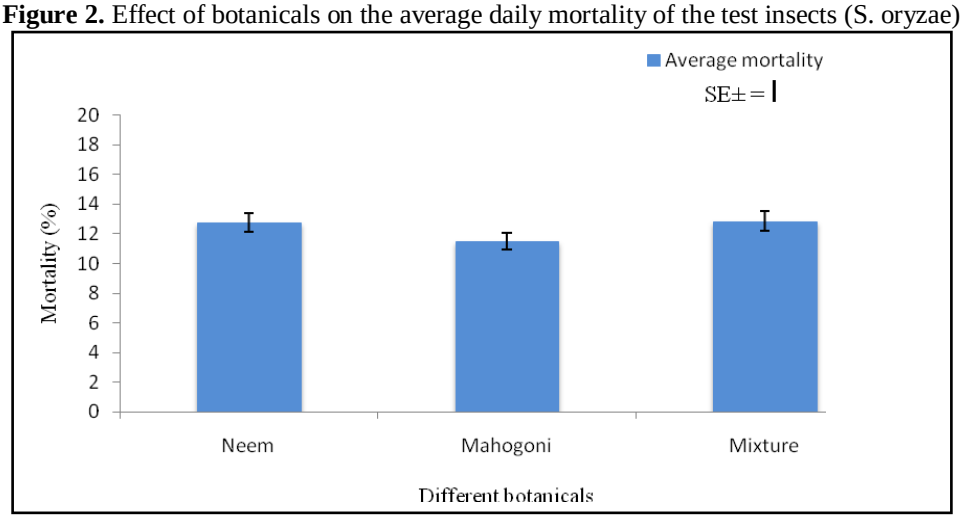
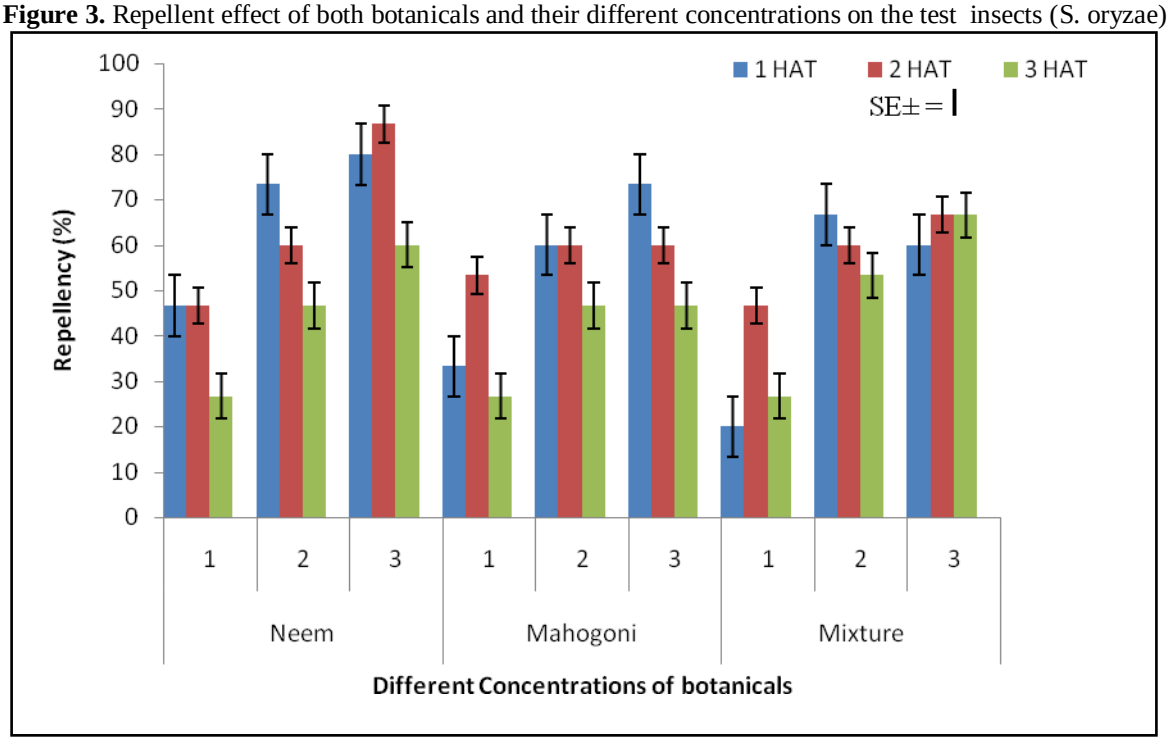


Figure 3. Repellent effect of both botanicals and their different concentrations on the test insects (S. oryzae)



HAT=Hour after treatment

Figure 4. Average per hour repellent effect of both botanicals and their different concentrations on the test insects (S. oryzae)