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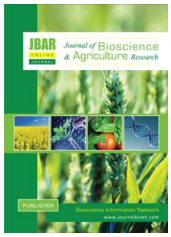

Evaluation of insecticidal activity of Ni (II) Cystine against the red flour beetle (*Tribolium casteneum*)

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ABSTRACT

The study was accomplished at the Crop protection and Toxicology Laboratory, Department of Zoology, Rajshahi University to evaluate the insecticidal activity of Ni (II) Cystine against red flour beetle. The main aim of this research is to evaluate the insecticidal activity of Ni (ii) cystine against the red flour beetle. Residual film bioassay and fumigation bioassay test were done and four doses of stock solution viz. S_1: 2 ml, S_2: 1 ml, S_3: 0.5 ml and S_4: 0.25 ml used in this study. Among them Residual film bioassay with S_1 showed strong activity against red flour beetle while fumigation test showed no activity on T. casteneum. Highest mortality (70%) found in film method and LD_{50} found 0.62 mg cm^{-2} in film bioassay test. Therefore, this study reveals that residual film method and Ni (II) cysteine has potential insecticidal activity against red flour beetle.

Key Words: *Film bioassay, Fumigation bioassay, Red flour beetle and Mortality*

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I. Introduction

Insects compete with man for their food supply and cause damage to field crops as well as to store products. Man has been fighting to protect his food from insect damage since his knowledge about their attack and losses to store grains. According to Hill (1978) losses of 25% or more may occur in tropical countries through insect attack after harvest. This reduction is further increased by the post harvests losses caused by the insects and other pests (Wright 1976). More than 2000 species of field and storage pests annually destroy approximately one third of worlds food production, valued at more than $100 billion among which highest losses (43% of potential production) occur in developing Asian countries (Ahmed and Graing, 1986). The red flour beetle (*Tribolium castaneum*) is one of most serious pests of stored products. Having the great economic importance of Tribolium, many studies on this pest have been dealt with its control. The control of insect pests by the application of chemicals has been known

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to store many years ago. Throughout the world an estimation of 4.1 thousand million pounds of pesticides is being applied annually (Turtle 1977) and of these 50% are used only for the protection of agricultural commodities. Chemical control method is the most potent one to obtain the maximum effectiveness within the minimum time and it is cost effective too. Except pesticide control method, no other control method has been established in Bangladesh so far. Resistance results in increased pesticide application frequencies, increased dosages, decreased yields, environmental damage and outbreaks of insect-borne human and veterinary diseases. Their presence in a stored product results in both contamination and substantial economic damage due to its loss and a decrease in nutritional value. Therefore, it is very important to reduce the economic losses due to red flour beetle (Burkholder and Faustini, 1991; Wilbur and Mills, 1985). So, the insecticidal activity of our test compound Ni (II) cystine has been evaluated against *Tribolium castaneum* by residual film bioassay and fumigation bioassay.

II. Materials and Methods

Insecticidal activity of Ni (II) cystine has been evaluated on *Tribolium castaneum* by residual film bioassay and fumigation bioassay method. Probit mortality of *Tribolium castaneum* was found to be 5.43731, 4.85612, 4.27493 and 3.69375 after 24 hours of surface film treatment with Ni (II) cystine complex at dose 2ml, 1 ml, 0.5ml and 0.25ml respectively. From these observation, calculated LD_{50} value have been found to be 0.6173 for *Tribolium castaneum* indicating significant insecticidal effect of Ni(II) cystine on these insects. Surface film method also used for determining the effect of Ni (II) cystine on *Tribolium castaneum.*

Culture of *Tribolium castaneum*

Different strains of *Tribolium castaneum* (Herbst) cultures are maintained for about 15 years in the Crop protection and Toxicology Laboratory, Department of Zoology, Rajshahi University. From the cultures standard local strain of the beetles was collected for the present study. Red flour beetles were sorted out to start a fresh culture. Earthen pots, Beakers (500 ml), Petri dishes, Camel hairbrush, Filter paper were used for maintaining the culture. All the equipment were kept in an oven for sterilization about six hours at 60°C. Mass cultures were maintained in earthen pot and subcultures in beakers with the food medium. The beakers were kept in an incubator at 30°C ± 0.5°C without light and humidity control. The cultures were checked in regular intervals and eggs and larvae were separated to increase properly. For continuously and huge supply of the beetle's sub-cultures were maintained in the incubator. The wheat flour was used as the food medium for *T. castaneum*. For each strain about 500 beetles were placed in a 500-ml beaker containing food medium and kept in an incubator at 30°C ± 0.5°C. The eggs were then transferred to Petri dishes (90 mm) and incubated at the same temperature. After 3-5 days larvae hatched out in that conditions. Newly hatched larvae were then collected with a fine pointed camel hair-brush and then shifted to the fresh food medium for culture. Then larval instars determine and larval cultures were maintained in an incubator at 30°C ± 0.5°C without light and humidity control. Newly formed adults were collected in a small beaker with the help of a camel hair brash.

Effect of Ni (II) cystine by surface film method on *Tribolium castaneum*

With four replications, different doses of Ni-cyst were applied as 2, 1, 0.5 and 0.25 mg ml and the mortality was subjected to probit analysis. Probit Analysis is a specialized regression model of binomial response variables. The regression is a method of fitting a line to the data to compare the relationship of the response variable (Y) to the independent variable (X). The dead beetles in Petri dishes after 24 hours treatment were shown in Figure 01.

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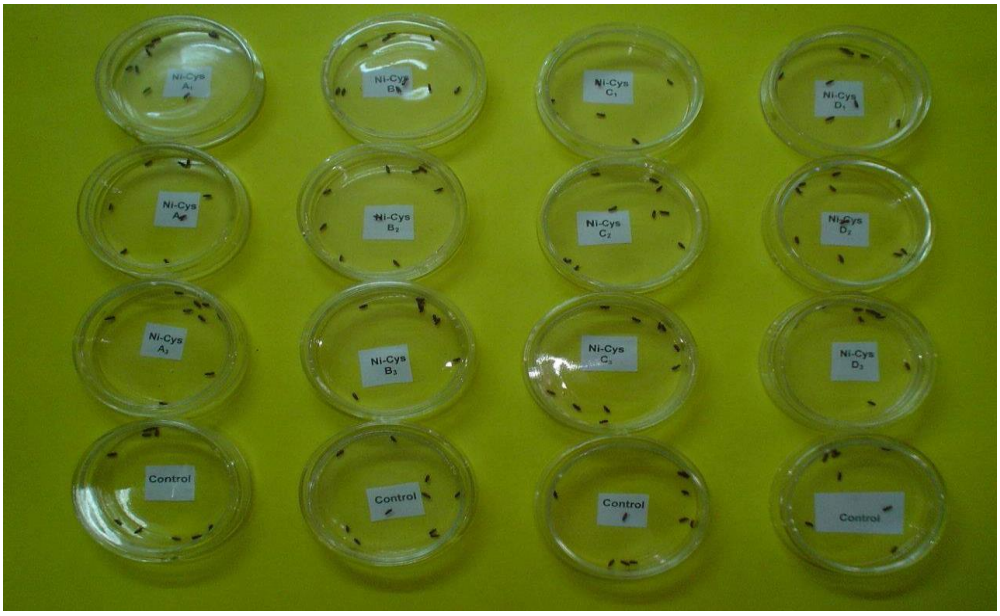

Figure 01. Dead beetle in the Petri dishes after 24hr of treatment.

Residual film bioassay and fumigation test

To carry this experiment by residual film method the adult red flour beetles were used. Ten beetles were used in each replication. The compound was weighed and diluted in alcohol to obtain doses in which mortality rate was between 10 to 90% for the 4 to 10 days old beetles. Four doses of stock solution viz. S_1:2 ml, S_2:1 ml, S_3:0.5 ml and S_4:0.25 ml used in this study. In each petridish10 beetles were inoculated and S_5: control experiment was maintained with alcohol. The mortality of the beetles was recorded after 24 hours of treatment.

The compound was tested for its fumigation activity to *T. castaneum*. For that two sets of experiments were done.

The mortality percentage was corrected using Abbott's formula (Abbott 1925):

$P_t = \frac{P_0 - P_C}{100 - P_c} \times 100$ Where, P_t = Corrected mortality (%) P_o= Observed mortality (%) and P_c=Control mortality (%)

The observed data then subjected to probit analysis (Finney 1947; Busvine 1971).

The probit analysis was used to compare the effect of Ni (II) cystine on *Tribolium castaneum* with drug λ-cyhalothrin. The LD_{50} value of Ni (II) cystine was found to be 0.62 mg/cm^{2}where as the LD_{50} value of known drug λ-cyhalothrin is 0.2416 which indicate the comparable insecticidal effect of Ni (II) cystine.

III. Results and Discussion

With four replications, different doses of Ni-cyst were applied as: 2 ml, 1 ml, 0.5 ml and 0.25 ml and the mortality was subjected to probit analysis. The probit mortality of Ni (II) cystine on adult *T. castaneum* after 24 hours of surface film treatment was shown in (Table 01) and number of insect killed (21), kill percentage (70), correct kill (69%), emported probit (5.5), exported probit (5.44), working probit (5.48) and final probit (5.43) found maximum in S_1 treatment while minimum from S_4 treatment.

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Table 01. Effect of Ni (II) cystine on probit mortality of adult *Tribolium castaneum* after 24 hours of surface film treatment

Treatment	No of insect	No of insect kill	No of insect kill (%)	Correct kill (%)	Emp. Probit	Exp. Probit	Wrk. Probit	Final probit
S1	30	21	70.0	69	5.50	5.44	5.48	5.43
S2	30	13	43.3	41	4.77	4.86	4.78	4.85
S3	30	8	26.3	24	4.29	4.27	4.28	4.27
S4	30	4	13.3	10	3.72	3.69	3.73	3.69
S5	30	1	3.30	_	_	_	_	_

Similar result was found in Ashok Kumar et al. (2016). However, in control (S_5) treatment showed no activity on correct kill percentage and on probit activity. Dubey et al. (2008) and Benzei et al. (2009) found no mortality and probit activity in control treatment, The LD_{50} was found to be 0.62 mg cm^{-2} after 24 hours of treatment (Table 02).

But no mortality of the beetle was observed and as such the compound offers no toxicity on fumigation (Table 03). Similar result was found in Mamun et al. (2009). Mamun 2009 reported in his study that from the probit results, it is clear that all the tested plants would be more or less effective for controlling red flour beetle, but neem will be the most effective one because the LD_{50} value of neem was 11.35% which was lowest than others.

Table 02. Mortality data and LD_{50} of Ni-cyst for surface film method to *Tribolium castaneum*

Test organism	Treatment	No. used	No. killed	Mortality (%)	LD_{50} / LC_{50}
T. castaneum (Surface film)	S_1	30	21	70	0.62 mg cm^{-2}
	S_2	30	13	43.3	
	S_3	30	8	26.7	
	S_4	30	4	13.3	
	S_5	30	1	3.3	

Table 03. Mortality data and LD_{50} of Ni (II) cystine for fumigation method to *Tribolium castaneum*

Test organism	Treatment	No. used	No. killed	Mortality (%)	LD_{50}/LC_{50}
Tribolium castaneum (Fumigation)	S_1	45	0	0	0
	S_2	45	0	0	
	S_3	45	0	0	
	S_4	45	0	0	

IV. Conclusion

From this experiment, we conclude that fumigation test compound Ni (II) cystine was not shown any activity to *Tribolium castaneum* while 2ml stock solution had strong activity against red flour beetle in bioassay test.

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