Detrimental Effects of Neem Seed on Different Life Stages of Red Flour Beetle, *Tribolium castaneum*

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Abstract: Fine Neem Seed Powder (NSP) was tested in three dozes, viz. 0.5%, 1.0% and 2.0% (w/w) for determination of its toxicity and detrimental effects on life stages of red flour beetle, *Tribolium castaneum.* Fifty gram treated and untreated (control) wheat was kept in glass jars and 10 pairs of the beetles were released in each. Mortality count was made after 72 hours. LD_{50} calculated as 1.841% NSP in wheat (w/w). NSP served as an Insect Growth Regulator (IGR). It was observed that at 0.5% dose, the number of larvae was not very different to control but the weight of larvae was lesser. At 1.0% dose, the number and weight of larvae were significantly reduced. At 2.0% dose, both the number and weight of larvae, pupae and adults were reduced remarkably. The insect growth inhibition was increased by increasing the dose of NSP.

Keywords: Neem seed powder, antifeedant activity, red flour beetle.

INTRODUCTION

Grains become infested by insect pests during storage rendering them unfit for human consumption. Red flour beetle, *Tribolium castaneum* (Herbst), is the most abundant and injurious pest of stored grains. It is one of the major pests of wheat in warehouses and flour mills at warm and tropical regions of the world, as reported by various researchers including Semple [1], Arnaud *et al.* [2], Zettler [3] and Trematerra & Sciarretta [4]. Several methods are being used to overcome the problem. Kostyukovsky [5] emphasized on the need for searching safe and natural management methods as alternative to pesticides, considering the increasing concern about health and environment. Utilization of natural plant products, *e.g.,* mixing of neem materials to save grains from insect pests is an old-age practice in some parts of the world. Natural products of plants have been tested by various researchers for their insecticidal properties against insect pests, such as, Dennis [6], Hossafay & Faisal [7], Emeasor *et al.* [8], Tooba *et al.* [9], Iqbal *et al.* [10], Sarwar & Sattar [11] and Sarwar *et al.* [12]. There is still need to search easily available bioactive plant materials for grain protection from insect pests during storage. All parts of neem, *Azadirachta indica,* have some biologically active components that exert detrimental effects on insect population. Neem derivatives are generally not hazardous to agro-ecosystem, as well as insect resistance is not developed like synthetic insecticides. Various researchers have worked on different aspects of *Azadirachta indica*. They include Meisner & Ascher [13], Naqvi *et al.* [14, 15], Jilani *et al.* [16], Schmutterer [17], Sharma [18], Xie *et al.* [19], Akhter *et al.* [20], Mordue *et al.* [21], Isman [22], Khan [23, 24], Ahmad *et al.* [25], Khan & Ahmad [26], Koul [27], Athanassiou *et al.* [28] and Iqbal [29]. However, there is a need for determination of more bio active plants for management of red flour beetle as identified by Wong [30]. This research was conducted to evaluate detrimental effects of neem seed powder (NSP) against red flour beetle, *Tribolium castaneum* (Herbst). The research may enrich the existing knowledge on effective, safe and environmental friendly protection of stored grains by the indigenous plants.

MATERIALS AND METHODS

Rearing Technique

Insects were reared in laboratory at 30˚C ± 1˚C temperature with relative humidity of 60% ± 5% on whole wheat grains as food medium in glass jars, covered with muslin cloth. Ten-day old adults were used for all the trials.

Test Neem Material

Fine neem seed powder (NSP) was used in all the trials. Neem seeds were collected and dried under shade. Dried seeds were ground in an electrical grinder to a fine powder of 30 meshes. This fine NSP was tested in three doses of 0.5%, 1.0% and 2.0% (w/w).

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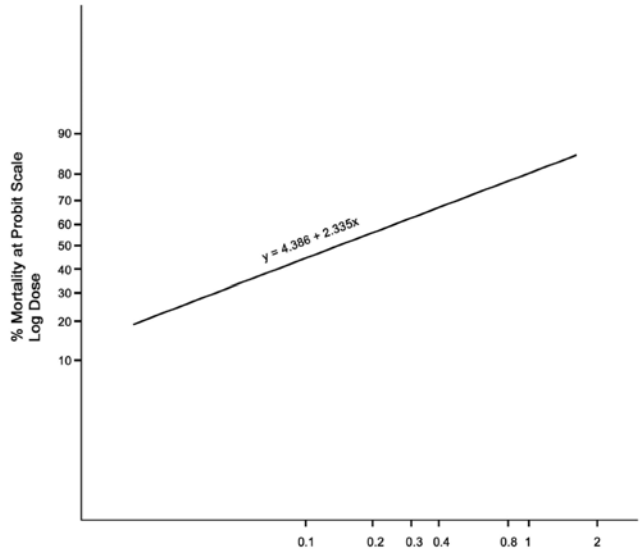

Figure 1: Probit Mortality curve of T castaneum.

Treatment Method

Fifty gram of treated and untreated (control) wheat was kept in 450-gm capacity jars. Twenty, 10-day old adults of red flour beetle, *Tribolium castaneum,* were released in each jar. Insects were removed from the jars after 72 hours and mortality was noted. All the trials were replicated five times. Observations were regularly recorded to check the growth of the insects. Total count and weight were measured after 15 days for larvae, 25 days for pupae and 40 days for adults. All the tests were performed under the same temperature and humidity. All the data were analyzed statistically.

RESULTS AND DISCUSSION

Fine NSP was tested in three doses of 0.5%, 1.0% and 2.0% (w/w) against adult red flour beetles. Mortality count was made after 24 hours of treatment. Adult mortality was calculated with Probit Analysis after Varely *et al.* [31]. Probit line is shown in Figure **1**. Probit regression equation calculated was Y = 4.526685 + 2.335113 (X – (6.150449E – 02)) whereas heterogeneity factor (Chi Square) was 8.330109 (Table **1**). Probit and Lethal Dose (LD) values are presented in Table **1**. The log LD_{50} at 72 hours treatment was found to be 1.84%; whereas, potential mortality was higher in 1% treated wheat. It could be probably due to the fact that higher dose because of high antifeedant effect prevented the organism to consume the toxic material. Contrarily, the moderate doses were consumed more and therefore caused higher mortality effect over the generation (Table **3**). Survival and effect of NSP on larval and pupal weights are shown in Tables **2** & **3**. It was found that NSP exerted weight reduction effect on larval, pupal and adult stages as average larval, pupal and adult weights were found to be 0.386 gm, 0.296 gm and 0.190 gm in control; 0.272 gm, 0.142 gm and 0.090 gm in 0.5% treated; 0.190 gm, 0.044 gm and 0.023 gm in 1.0% treated; and 0.112 gm, 0.022 gm and 0.002 gm in 2% treated, respectively. These results

Table 1: Toxicity of NSP Against *Tribolium castaneum* (Adults) after 72 Hours

Compound	Conc. (%)	Dose (gm)	Mortality (%)	SD	SE	Mean ± SE x Sig. value at 95% confidence limit
Control	0.00	0.00	02.00	2.70	1.22	2±2.4
NSP	0.50	0.25	12.00	5.70	2.55	12±5.0
NSP	1.00	0.50	24.00	8.22	3.67	24±7.2
NSP	2.00	1.00	56.00	9.62	4.30	56±8.4

Regression equation: Y = 4.526685 + 2.335113 (X – (6.150449E – 02)).
Heterogeneity factor: Chi Square χ^2 = 8.330109.

Lethal Dose		Standard Error	Fudicial Limits (95%)	
LD_{10}	0.521	0.037	0.443	0.613
LD_{30}	1.102	0.022	1.007	1.208
LD_{50}	1.841	0.025	1.632	2.077
LD_{70}	3.074	0.410	2.543	3.717
LD_{90}	6.504	0.068	4.781	8.849
LD_{99}	18.318	0.107	11.349	29.565

Table 2: Effect of Neem Seed Powder on Immature Stages of *Tribolium castaneum*

Conc. (w/w)	Replication	Larvae		Pupae		Adults	
		Count	Wt. (gm)	Count	Wt. (gm)	Count	Wt. (gm)
Control	1st	150.00	0.39	112.00	0.24	98.00	0.141
	2nd	128.00	0.21	105.00	0.22	102.00	0.181
	3rd	161.00	0.45	98.00	0.19	89.00	0.190
	4th	165.00	0.37	147.00	0.38	120.00	0.210
	5th	210.00	0.51	193.00	0.45	149.00	0.230
	Total	814.00	1.93	655.00	1.48	558.00	0.952
0.5%	1st	115.00	0.28	41.00	0.10	31.00	0.065
	2nd	142.00	0.30	79.00	0.17	48.00	0.079
	3rd	171.00	0.39	82.00	0.15	59.00	0.093
	4th	143.00	0.21	67.00	0.13	58.00	0.103
	5th	125.00	0.18	78.00	0.16	62.00	0.110
	Total	696.00	1.36	347.00	0.71	258.00	0.450
1.0%	1st	72.00	0.21	48.00	0.06	22.00	0.041
	2nd	102.00	0.24	40.00	0.06	16.00	0.010
	3rd	65.00	0.18	14.00	0.02	4.00	0.002
	4th	93.00	0.21	55.00	0.10	26.00	0.043
	5th	55.00	0.11	21.00	0.03	13.00	0.018
	Total	387.00	0.95	178.00	0.27	81.00	0.114
2.0%	1st	16.00	0.05	8.00	0.01	1.00	0.001
	2nd	28.00	0.09	10.00	0.04	3.00	0.004
	3rd	7.00	0.02	5.00	0.01	1.00	0.001
	4th	25.00	0.09	8.00	0.02	2.00	0.002
	5th	31.00	0.12	12.00	0.03	5.00	0.005
	Total	107.00	0.37	43.00	0.11	12.00	0.013

show an increase in weight reduction with in NSP concentrations. This phenomenon could be due to the antifeedant activity of neem as indicated by Sharma *et al.* [32] and Khan [23]. Weight reduction could also be due to growth retardation and difference in metabolism. Growth retardation by neem derivatives has been reported by Sharma [18] and Khan & Ahmed [26]. The present findings on neem against the beetle are in line with the previous reports. As indicated before, NSP exerted acute mortality in adults. Killing potential (k factor) for treated medium reared beetles observed was 0.49 for 0.5%, 0.99 for 1% and 1.8 for 2% as compared to the untreated (control) where k factor was found 0.16. Similar findings on toxicity of neem seed against *T. castaneum* and *T. cofusum* are reported by Ahmad *et al.* [25] and Khan & Ahmad [26]. The present findings on detrimental effects of neem seed powder may be a beneficial addition in the work (on plant products) conducted by earlier researchers. Some researchers evaluated plants powder for their effectiveness against stored grain pests. Al-Moajel [33] prepared powders from parts of different plant species and found (62%) mortality at 6% concentration against *Trogoderma granarium*; Silva *et al.* [34] evaluated up to 99.3% mortality of *Sitophilus zeamais* by powdered plants; Emeasor *et al.* [8] found plant seed powders very toxic at 1 and 2 gm/ 20 gm seeds against *Callosobruchus maculates*. The results of the study indicated that the neem seed has good biological activity against insect and fine NSP may be used for stored grain treatment as dusting and bag treatment as well. The use of neem seed powder (NSP) would not need any standardization; however, Sivakumar *et al.* [35] advised use of appropriate machinery, *e.g.,*

Table 3: Killing Factor and Potential Mortality Effects on Various Life Stages of *Tribolium castaneum* Reared on Neem Seed Treated Media

	Control *					0.5% **							0.1% ***							0.2% ****						
	NL	SI_{LP}	NP	SI_{PA}	NA	NE	SI_{EL}	NL	SI_{LP}	NP	SI_{PA}	NA	NE	SI_{EL}	NL	SI_{LP}	NP	SI_{PA}	NA	NE	SI_{EL}	NL	SI_{LP}	NP	SI_{PA}	NA
Population	814		715		558	814		696		347		258	814		387		178		81	814		107		43		12
Dying Interval		99		159			118		349		89			427		209		97			707		64		31	
Mortality (%)		12.2		19.3			14.5		42.9		10.9			52.5		25.7		1.0			87.0		7.86		3.8	
Successive Mortality (%)		12.2		22.0			14.5		50.0		26.0			52.5		54.0		54.5			87.0		60.0		72.0	
Successive Survival (%)		87.8		78.0			85.5		50.0		74.0			47.5		46.0		45.5			13.0		40.0		28.0	
Fraction Survival		0.88		0.78			0.86		0.50		0.74			0.475		0.460		0.455			0.13		0.40		0.28	
Log Population	2.91		2.85		2.75	2.91		2.84		2.54		2.41	2.91		2.59		2.25		1.91	2.90		2.03		1.63		1.08
K-Value		0.06		0.10			0.06		0.30		0.13			0.31		0.34		0.34			0.87		0.40		0.55	

	Σ Mortality	Σ Mortality (%)	Generation Survival	K-factor
*	258	31.5	0.680	0.16
**	556	68.3	0.320	0.49
***	733	90.11	0.099	0.99
****	802	98.66	2.170	1.82

NE: Number of eggs.
NL: Number of larvae.
NP: Number of pupae.
NA: Number of adults.
SI_{EL}: Standard interval between eggs to larvae.
SI_{LP}: Standard interval between larvae to pupae.
SI_{PA}: Standard interval between pupae to adults.

decorticator, seed/kernel crusher and pulverizer that may be needed for processing plants at village level.

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