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RESEARCH ARTICLE **OPEN ACCESS**

TOXIC POTENTIAL OF NEEM STEM ASH POWDER (*AZADIRACHTA INDICA*, A. JUSS) AGAINST *CALLOSOBRUCHUS CHINENSIS* INFESTATION (BRUCHIDE; COLEOPTERA) ON THE COWPEA (*VIGNA UNGUICULATA*) SEEDS

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ABSTRACT

Every year insect pests damaging pulse crops cause heavy economic loss. These pulses during storage are attacked by the most destructive pest *Callosobruchus chinensis*. Neem in different ways and in different formulation found effective against stored pests. Hence study was conducted to observe the toxic potential of Neem stem ash powder against the stored pests of cowpea seeds. The studies were conducted to evaluate the efficacy of the neem stem ash powder on *C. chinensis*, and to analyze the protective action of neem products on the physical and biological parameters (colour, hardness, number of boreholes/seed damage, percent weight loss, percent protection of seeds and the number of eggs laid, adult emergence, percent adult mortality, percent reproductive success and growth index) of the infested seeds. In the present investigation, after application of NLP and NSAP of different doses, the results showed significant reduction in the adult emergence in treated seeds. From the statistical analysis of *C. chinensis* it was observed that all the treatments were significantly superior to control in minimizing the pest. In the present investigation, after application of NLP and NSAP, the results showed a significant reduction in the insect population with increasing percent adult mortality in treated grains.

Key words: Toxic potential, neem stem-ash, cowpea seeds, *Callosobruchus chinensis.*

INTRODUCTION

Pulses play a vital role in relieving protein malnutrition in many areas where animal protein cannot be afforded. Insect pests cause heavy loss of stored grain, particularly in tropical farms. Stored product insect pests cause loss directly by reducing dry weight, germination, nutritional value, or the grade of harvested grain. The Food and Agricultural organization of the United Nations, estimates that 5-10% of harvested grain is lost in storage, the loss being higher in some developing countries (Hall, 1970). Pulses, being a rich source of protein (22-30%) offer the most practical means of solving malnutrition in our country, where the majority of people are vegetarian. India produces around 12.65 million tonnes of different pulses per year but nearly 8.5 percent of the same is lost during post harvest handling and storage (Agarwal *et al*., 1988).

Parkin and Bills (1995) reported that the major insect pests infesting stored pulses belong to the Bruchidae family. They are prolific in their breeding and rapidly cause a serious reduction in nutrition, viability values of the pulses. *Callosobruchus* species are cosmopolitan in habit and its degree of damage depends upon the humidity, temperature and abundance of food material in the stores and also the moisture content of the grain at the time of storage.

Sankar and Rattan (1995) stated that the continuous, imbalanced and indiscriminate use of chemical fertilizers alone may create problems to soil productivity particularly degradation of soil's physical and chemical properties. Mann *et al*. (2002) revealed the increasing concern about pesticide accumulation in the environment which has stimulated the search for natural compounds that could replace synthetic insecticides in insect pest control.

Pradhan *et al.* (1973) reported the repellent properties of some neem products. Wulf (1991) studied that azadirachtin is a natural environmental compatible product, which could be promising for pest control. Its extracts are known to affect more than 200 species of insects many of which are resistant to an inherently difficult to control with conventional pesticides.

Various products of neem have been used since long for the control of various pests of stored grains. Wood ash has been reported to be effective in the control of several storage pests by offering mechanical protection especially when mixed with the seeds (Head lee, 1924). Such an admixture of seeds with clay, ashes, talc or sand formed on the earliest recommendations for the control of bruchids in beans (Subramanian, 1953; Deay and Amos, 1936 and Lever, 1941).

So the present study was carried out to evaluate the efficacy of neem stem powder on the pulse beetle, *Callosobruchus chinensis* which is a common pest in a number of stored grains.

Objectives of the present study

i) To evaluate the efficacy of the neem stem ash powder on *C. chinensis.*

ii) To study the use of certain products from neem plant (*Azadirachata indica)* for their protective action on stored pulse cowpea against the infestation of the pulse beetles.

iii) To analyze the protective action of neem products on the physical and biological parameters (colour, hardness, number of boreholes/seed damage, percent weight loss, percent protection of seeds and the number of eggs laid, adult emergence, percent adult mortality, percent reproductive success and growth index) of the infested seeds.

MATERIAL AND METHODS

In the present experiment, the selected stored pest was *C. chinensis* which was widely distributed all over the world. It was commonly called cowpea bruchids.

C. chinensis is the most destructive pest of stored grains. The larvae bore into the seeds. Infestations usually originate from farm stores but the adult beetles can fly for up to about half a mile.

Neem stem was collected from local areas. The dried neem stem was burnt out and powdered. The ash powder was sieved using 1mm mesh so as to remove the large particles. Neem has several functions like antifeedant, repellent, deterrent, oviposition inhibitor, growth and metamorphosis inhibitor, and affects the fecundity and egg-sterility. It is also used by human beings for different purpose.

Color

Seed color was noted from the appearance qualitatively.

Hardness of the seed

Hardness of the seeds was found out by pressure extertion method using a thumb pressure to find out the cracking point time. The time taken to cause the cracking of the grain was noted to find out the relative hardness.

The seed colour and hardness of the seed was found out by the method followed by Avidov *et al*. (1965) and Gokhale and Srivastava (1975).

Damaged seeds/percent protection of seeds

The percent seed damage was calculated by counting the damage and undamaged seeds (by counting the number of boreholes) in each replicate on comparing to the control. Percentage of the protection of pulses was calculated by the formula used by Doharey et al. (1990) by counting the number of boreholes of control and treated seeds.

$$\text{Percent protection of pulses} = \frac{\text{Control - treatment}}{\text{Control}} \times 100$$

Percent weight loss

The percent loss in weight due to insect damage was calculated by using the following formula of Dabi et al. (1979).

$$\text{Percent weight loss} = \frac{\text{Initial weight of grain - final weight of grain (afer 30 days)}}{\text{Initial weight of grain}} \times 100$$

Biological parameters

Number of eggs laid

For the study of ovipositional performance, 100 weighed seeds of cowpea were kept separately in plastic containers arranged in a circular fashion in a glass trough along with control and 5 pairs of newly emerged beetles were introduced. The mouth of the trough was covered with double – fold muslin cloth fastened with rubber band. This experiment was replicated thrice. The released insects were removed with a fine camel hair brush after 72 hours with the expectation of maximum oviposition during this period and the number of eggs laid on each variety was counted visibly (Sanjay Sharma, 1999). This experiment was kept continued for 30 days to observe the adult emergence.

Number of adults emergence

Observation on number of adults emerged was recorded every alternate day, till no further emergence could be seen.

Percent adult mortality

After the emergence of adults, number of adults died were observed by visual counting. The percent adult mortality was calculated by using the formula of Prabha and Sehgal (1990).

$$\text{Percent adult mortality} = \frac{\text{Number of adults died}}{\text{Number of adults emerged out}} \times 100$$

Percent Reproductive Success

Percent reproductive success (RS) was calculated by the formula used by Prabha and Sehgal (1990).

$$\text{Percent reproductive succes} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

Growth index

The growth index was also calculated by the formula used by Prabha and Sehgal (1990).

$$\text{Growth index} = \frac{\text{Percent reproductive success}}{\text{Developmental period}}$$

The results obtained in all the above parameters were analyzed statistically (Students 't' test) followed by Mishra and Mishra (1989) to find out the level of significance of the treatment by comparing with that of the control.

RESULTS AND DISCUSSION

The extracts from neem, *A. indica*, have been reported to produce diverse biological effects on insects; antifeedant (Doharey and Singh, 1989), growth disruptor (Sharma *et al.*, 1980), oviposition deterrent (Singh and Srivastava 1983), toxic in nature (Singh *et al.*, 1988) etc., of these antifeedant activity of neem is considered to be very important.s

From the current study, it was obvious that NLP were observed to be more effective checking seed damage, percent weight loss, percent protection of seeds, egg laying, adult emergence, percent adult mortality, percent reproductive success and growth index than the control (Tables 1-6). NSAP treatment proved to be lesser effective on the above mentioned parameters than NLP treatment. The two neem treatments showed an increase in their rate of checking action with respect to the increasing dosage in the following order; NLP > NSAP. This may be due to the presence of a chemical called azadirachtin which has been identified as the key compound that works as an insect feeding deterrent and also as an inhibitor of ecdysis and growth in a larger quantity in NLP. Also the burning of stem to obtain stem ash powder might have altered the chemical nature of these chemicals to a certain extent. The research conducted by the United States Department of Agriculture has shown azardirachin protective action against infestation of insects on grains and pulses.

This is a tetranortriterpenoid compound. A severe antifeedant activity was reported to occur by seed kernel powder by Butterworth and Morgan (1971), leaf and bark (Kraus *et. al.,* 1987) and in the seed coat and seed oil (Rembold *et. al.,* 1987). This may be due to that seed kernel powder might have exhibited higher gustatory repellent property than leaf and seed coat powders.

In the present study, results obtained from the egg laying parameter revealed that, the total number of eggs laid per seed was more in the treated cow pea seeds at all the three different concentrations. The influence of colour of chickpea seeds on the oviposition of *C. chinensis* was also reported by Khare and Johari (1984). The emergence of adult was greatly reduced with the increase of the hardness of the grains. The grain damage had been influenced by hardness to a greater extent and the weight loss of the seed was found to be inversely proportional to the hardness of grain. Similar results were recorded by Rout *et. al.* (1976) and Cogburn (1974) who observed the hardness of grains provided an inverse relationship with the pest infestation. Further, Singh and Sharma (2002) also reported that the increase in hardness had decreased the growth and development of *C chinesis* on pea.

The average number of boreholes / seed damage observed in cow pea seeds at different concentrations of NLP and NSAP at different interval periods, showed a significant reduction in boreholes/ seed damage after 10 days exposure compared to 20 and 30 days exposures and the control. The degree of boreholes/seed damage at different intervals in both the seeds and treatments showed the degree of loss of weight in the seeds which depend upon the repellent property of the neem seed. The treatments at 0.5mg/100gm and 0.7mg/100gm seeds concentrations of NLP and NSAP have afforded effective protection against bruchid infestation in cow pea seeds.

In the present study, the loss in weight was found to be in negative relationship with the hardness of the seeds (i.e. cowpea showed lesser weight loss of seeds). Similar work was also studied by Singh Umrao and Sharma (2002). The protective potential was superior in NLP treatment than NSAP. The results find support from Jaipal *et. al.* (1984) who demonstrated that neem leaf extracts were effective for the control of *Rhyzopertha dominica.*

The number of eggs laid on seeds were ranged from 0.9 to 4.9. The response of the two powders at three different concentrations regarding egg laying on seds was found to be statistically significant. This indicates that the rough surfaced (cowpea) texture was found to be more preferred by the pests.

In the present study, a decrease in adult emergence was noticed at higher concentration (0.7 mg/100 gm seeds) in NLP treatment than in the other two concentrations as well as in the control. It is clear, from the present study, that NLP can be regarded as superior over NSAP. The adult emergence was decreased with increasing doses and also related to days of exposure.

In the present investigation, after application of NLP and NSAP of different doses, the results showed significant reduction in the adult emergence in treated seeds. From the statistical analysis of *C. chinensis* it was observed that all the treatments were significantly superior to control in minimizing the pest. In the present investigation, after application of NLP and NSAP, the results showed a significant reduction in the insect population with increasing percent adult mortality in treated grains. All the treatments were statistically analyzed and proved to be significant and superior in control in minimizing the pest infestation. NLP is recorded to be highly significant at 0.7mg/100gm seeds concentration of cowpea seeds and manifested highest toxicity, and treatment containing 0.3mg/ 100 gm seeds concentration showed least damage.

Table 1. The number of bore holes / seed damage by *Callosobruchus chinensis* in treated Cow pea seeds with different concentrations of NLP & NSAP after 10, 20 and 30 days exposure.

Period	**Cow pea**					
	10 Days		**20 Days**		**30 Days**	
Concentration (mg / 100gm seeds)	**NLP**	**NSAP**	**NLP**	**NSAP**	**NLP**	**NSAP**
0.3 Mean ± 't' value	Nil	Nil	Nil	Nil	2.8 6.60^{***}	3.4 2.61^{**}
0.5 Mean ± 't' value	Nil	Nil	Nil	Nil	2.5 7.20^{***}	2.7 6.57^{***}
0.7 Mean ± 't' value	Nil	Nil	Nil	Nil	1.8 12.60^{***}	2.1 12.72^{***}
Control Mean ±	Nil	Nil	Nil	Nil	3.9	3.9

Significant level

$p < 0.05^{*}$ $p < 0.01^{**}$ $p < 0.001^{***}$

Table 2: Percent weight loss in cow pea seeds infested by *Callosobruchus chinensis*after 30 days treatment with NLP & NSAP

Concentration (mg / 100gm seeds)	**Cow pea**					
	NLP			**NSAP**		
	Initial weight	**Final weight**	**Weight loss (%)**	**Initial weight**	**Final weight**	**Weight loss (%)**
0.3 't' value	100	88.3	11.7 3.2^{***}	100	86.1	13.9 1.6^{*}
0.5 't' value	100	90.9	9.1 4.6^{***}	100	88.4	11.6 3.2^{**}
0.7 't' value	100	95.8	4.2 5.8^{***}	100	92.3	7.7 4.3^{***}
Control	100	82.8	17.2	100	82.8	17.2

Significant level

$p < 0.05^{*}$ $p < 0.01^{**}$ $p < 0.001^{***}$

Table 3. The number of eggs laid and adult emergence of *Callosobruchus chinensis* in NLP and NSAP treated Cow pea at different concentrations after 10, 20 and 30 days exposure.

Period	**10 Days**				**20 Days**				**30 Days**			
Concentration (mg / 100 gm seeds)	**NLP**		**NSAP**		**NLP**		**NSAP**		**NLP**		**NSAP**	
	No. of eggs laid	**No. of adults emerged**	**No. of eggs laid**	**No. of adults emerged**	**No. of eggs laid**	**No. of adults emerged**	**No. of eggs laid**	**No. of adults emerged**	**No. of eggs laid**	**No. of adults emerged**	**No. of eggs laid**	**No. of adults emerged**
0.3 mean ± ‘t’ value	2.9 4.02^{***}		3.3 1.98^{*}	Nil	2.9 4.02^{**}	Nil	3.3 1.98^{*}	Nil	3.7 6.57^{***}	2.4 4.33^{**}	4.3 3.28^{**}	2.9 1.75^{*}
0.5 mean ± ‘t’ value	2.3 5.96^{***}	Nil	2.7 4.37^{***}	Nil	2.3 5.96^{***}	Nil	2.7 4.37^{***}	Nil	3.1 12.72^{***}	1.9 7.83^{***}	3.8 4.91^{***}	2.3 4.91^{***}
0.7 mean ± ‘t’ value	1.7 8.34^{***}	Nil	2.1 7.60^{***}	Nil	1.7 8.34^{***}	Nil	2.1 7.60^{***}	Nil	2.4 13.06^{***}	1.2 10.43^{***}	3.0 10.58^{***}	1.6 7.79^{***}
Control mean ±	3.8	Nil	3.8	Nil	3.8	Nil	3.8	Nil	4.9	3.4	4.9	3.4

Significant level

$p < 0.05^{*}$ $p < 0.01^{**}$ $p < 0.001^{***}$

Table 4. Percent of adult mortality of *Callosobruchus chinensis* in treated cow pea seeds with different concentrations of NLP and NSAP after 10, 20 and 30 days exposure.

Period	**Cow pea**					
	10 Days		**20 Days**		**30 Days**	
Concentration (mg / 100 gm seeds)	**NLP**	**NSAP**	**NLP**	**NSAP**	**NLP**	**NSAP**
0.3 (%)	Nil	Nil	Nil	Nil	35.71	22.22
0.5 (%)	Nil	Nil	Nil	Nil	75	38.46
0.7 (%)	Nil	Nil	Nil	Nil	100	66.66
Control (%)	Nil	Nil	Nil	Nil	4.54	4.54

Table 5. Percent reproductive success of *Callosobruchus chinensis* in treated Cow pea seeds with different concentrations of NLP and NSAP after 10, 20 and 30 days exposure.

Period	**Cow pea**					
	10 Days		**20 Days**		**30 Days**	
Concentration (mg / 100 gm seeds)	**NLP**	**NSAP**	**NLP**	**NSAP**	**NLP**	**NSAP**
0.3 (%) 't' value	Nil	Nil	Nil	Nil	64.86 2.12^{*}	67.44 1.03^{NS}
0.5 (%) 't' value	Nil	Nil	Nil	Nil	61.29 3.80^{***}	60.52 4.72^{***}
0.7 (%) 't' value	Nil	Nil	Nil	Nil	50.0 10.35^{***}	53.33 7.76^{***}
Control (%)	Nil	Nil	Nil	Nil	69.38	69.38

Significant level

NS = Not significant $p < 0.05^{*}$ $p < 0.01^{**}$ $p < 0.001^{***}$

Table 6. The growth index of *Callosobruchus chinensis* in treated cow pea seeds with different concentrations of NLP and NSAP after 10, 20 and 30 days exposure.

Period	**Cow pea**					
	10 Days		**20 Days**		**30 Days**	
Concentration (mg / 100 gm seeds)	**NLP**	**NSAP**	**NLP**	**NSAP**	**NLP**	**NSAP**
0.3 Mean ± 't' value	-	-	-	-	1.96 0.88^{NS}	2.04 2.25^{*}
0.5 Mean ± 't' value	-	-	-	-	1.85 1.19^{NS}	1.83 9.17^{***}
0.7 Mean ± 't' value	-	-	-	-	1.51 7.23^{***}	1.61 5.33^{***}
Control Mean ±	-	-	-	-	2.10	2.10

Significant level

NS = Not significant $p < 0.05^{*}$ $p < 0.01^{**}$ $p < 0.001^{***}$

CONCLUSION

From the results obtained, it may be concluded that seeds of cowpea can be effectively protected from the damage of *Callosobruchus chinensis* by neem stem ash powder.

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