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BIOACTIVE NEEM LEAF POWDER ENHANCES THE SHELF LIFE OF STORED MUNGBEAN GRAINS AND EXTENDS PROTECTION FROM PULSE BEETLE

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ABSTRACT

A laboratory experiment was conducted to evaluate the protective efficiency of bioactive neem leaves powder (NLP) (Azadirachta indica) against pulse beetle (Callosobruchus chinensis) in stored mungbean grain and prolonging effect on its shelf life. A two factorial completely randomized design (CRD) with three replications was used having four levels of NLP (0, 0.5, 1.0 and 1.5 mg per 100 g seeds) and four storage durations (40, 60, 80 and 100 days). Main effect of NLP and storage duration showed significant differences for number of eggs laid, total progeny, adult mortality, % grain damaged and % weight loss, whereas non-significant differences were observed for their interactions for mentioned parameters. NLP applied @ of 1.5mg/100g seeds decreased no. of eggs laid by 36%, total progeny by 38%, % grain damage by 46%, % weight loss by 53% and increased adult mortality by 62% over control. Generally with the increase in storage duration, no. of eggs laid, total progeny, adult mortality, % grain damaged and % weight loss increased and their values ranged from 101.20-120.69 for no. of eggs laid, 73.50-86.58 for total progeny, 11.71-53.49 for adult mortality, 13.55- 24.45 for % grain damaged and 14.27- 30.65 for % weight loss from 40-100 days of storage duration. These findings suggest that bioactive NLP has a strong detrimental effect on pulse beetle in mungbean, thus it can maintain the quality and may enhance shelf life of mungbean seeds. Therefore, neem leaf powder coating can be used for safe management of pulse beetle repulsion during post-harvest storage of grains/seeds in different crops as an alternative to synthetic chemicals application.

Keywords: Bioactive, mungbean, neem leaves powder, pulse beetle.

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INTRODUCTION

At least 5% of the world production of all cereal grains is destroyed by insect pests during storage in the farms, warehouses and elevators after being harvested (Rathore and Sharma, 2002). Among them, pulse beetle *(Callosobruchus chinensis)* is a serious pest of leguminous stored seeds or stored pulses (Rathore and Sharma 2002). The pluse beetle, is a cosmopolitan insect pest of mung bean. It is a field -to-store pest as its infestation of mungbean often begins in the field as the mature pods dry (Huignard *et al.,* 1985; Sathyaseelan *et al.,* 2008) and when such seeds are harvested and stored, the pest population increases rapidly and results in total destruction within a short duration of 3-4 months (Rahman and Talukder, 2006). It multiplies very rapidly in storage (Ouedraogo *et al.,* 1996) and reported 8.5% loss in pulses during post-harvest handling and storage in India. Although effective control of beetle is possible with chemical insecticides like fumigants and dusts, but they pose possible health hazards to warm-blooded animals and a risk of environmental contamination. Pest also develops resistance to these chemicals after some time (Doris 1996). Thus the use of plant products is considered an important component of insect pest management because of their economic viability and eco-friendly nature. Insecticidal plants are effective alternative for chemical insecticides to reduce pesticide load in the environment (Radha and Susheela, 2014). Different compounds are recognized in number of plant species that possess some activity against pests (Harborne, 1998).

Botanicals have been used from a very long time by many farmers in the world from decades to control stored insect pests (Araya and Emana, 2009). It was inferred that among the botanicals the neem leaf powder play an essential role in controlling the pulse beetle infection in stored seeds. Neem contains several active constituents called limonoids among which, azadirachtin, salanin, nimbin, meliantriol are the major components. Many of these limonoids possesses insecticidal, ovicidal, antifeedancy, growth regulatory, sterilizing adults and repellency against many insects and storage pests (Akou-Edi, 1984; Makanjuola, 1989; Schmutterer, 1990).

Mungbean (*Vigna radiata*) is an important short-duration pulse crop in Pakistan. It is grown in Kharif as well as in spring season

(PARC). In terms of mungbean's cultivated area and its production, Punjab is the leading province followed by Khyber-Pukhtunkhwa, where it is largely grown in southern part mostly including D.I. Khan, Karak and Bannu districts (MINFA, 2013). Like other pulses or leguminous crops its seeds/grains are highly vulnerable to insect pest's damage during storage conditions and cause huge economic loss to farmers in terms of lowering the produce quality and possible shortage of food (Damon *et al.*, 2007). Therefore the current experiment was conducted to evaluate the potentials of bioactive neem leaf powder application as safe alternative of synthetic chemicals to eliminate the occurrence of pulse beetle and destruction caused by it during post-harvest storage in mungbean grains/seeds.

MATERIALS AND METHODS

A laboratory experiment was conducted in the Department of Plant Protection, the University of Agriculture, Peshawar, Pakistan during winter 2013 to study the efficacy of neem leaf powder (NLP) at various storage durations in controlling pulse beetle in mungbean. Two factors factorial completely randomized design with four replications was used for the experiment. There were four levels of NLP treatment i.e, 0, 0.5, 1.0 and 1.5 mg 100^{-1} g seeds, and four storage durations for 40, 60, 80 and 100 days at 28 ± 2°C and 70 ± 5% R.H (relative humidity). Mungbean grains were disinfested at 60 ^{0}C for 20 minutes and were placed in 48 cylindrical jars @ of 100 gram per 250 ml jar. Neem leaves were collected from grocery shop at Rawalpindi, sun dried in order to completely avoid the effect of moisture and grinded and sieved through 1 mm mesh sieve to get fine leaf powder. The desired doses of NLP were poured and the jars (10 cm in diameter and 30 cm heighted) were shaken for five minutes to uniformly coat the seeds. After 4-6 hours of treatments application, five pairs of beetles obtained from Nuclear Institute of Food and Agriculture (NIFA) Peshawar, Pakistan were introduced into each jar. The tops of the jars were then covered with perforated led to facilitate aeration and prevent escape of beetles (Satyavir, 1983).

Data were recorded on number of eggs laid for which ten grains were randomly selected from each replication and eggs laid on those grains were counted. At the end, their average was calculated to determine number of eggs per grain in each jar. For data on total progeny, total number of adults emerged in each treatment was recorded and regarded as the total number of progeny. Adult mortality data were recorded by counting total number of adult died in each treatment and regarded as the total number of adult mortality, data on percent grain damage were recorded by dividing total number of damaged grains by total number of grains and multiplied with 100 and data on percent weight loss were recorded using the following formula:

$$\text{Weight loss (\%)} = \frac{\text{Weight of the control} - (\text{Weight of sound} + \text{damaged grains})}{\text{Weight of the control}} \times 100$$

Data obtained were statistically analyzed by using statistical software STATISTIX 8.1 and means were compared by using least significant difference test (LSD) (steel and torrie, 1980).

RESULTS AND DISCUSSION

Numbers of eggs laid

Significant effects were observed for different levels of neem leaf powder (NLP) and storage duration on number of eggs laid by pulse beetle in mungbean (Table-1). Maximum number of egg laid of 138.24 per 100 g grains was recorded for control whereas minimum mean number of eggs laid of 88.75 per100g grains was recorded for 1.5 mg NLP 100 g^{-1} seeds. It was evident from result that with the increase in NLP rate the number of eggs laid was decreased. It might be due to high toxic effect of NLP at higher concentration. Same results were also obtained by Olaifa and Erhun 1998 who found that higher concentration of the powder of neem significantly reduced the oviposition. Similarly maximum mean eggs laid of 120.69 per 100g grains were recorded for storage duration of 80 days; however, it was statistically at par to 116 per 100 grains of 100 days. Storage duration of 60 days showed eggs laid of 109 per 100 g grains that were significantly lower than that of eggs laid at 80 and 100 days, while higher than that of 101 eggs laid per 100 g grains at 40 days. It was also evident from result that with the increase of storage duration number of eggs laid increased. It might be due to accumulation of unhitched eggs from previous generation and due to decrease in lethal/toxic effect of NLP with passage of time. Our results are in line with findings of Chinwada and Giga (1993) who reported that neem products were very effective against pulse beetles till sixteen weeks to reduce oviposition and percent eggs hatching. Non-significant differences were observed for interactive effect of NLP and storage duration for number of eggs laid.

Developmental period

Neem leaf powder (NLP) significantly affected developmental period of pulse beetle in mungbean at varying storage duration (Table-1). Highest developmental period of 42.91 days was recorded for NLP applied @ of 1.5 mg 100 g^{-1} grain. Minimum mean developmental periodof 32.25was recorded for control. This data indicated that with the increase in NLP concentration, developmental period increased which might be due to high toxic effect of NLP at higher concentration. Same results were also obtained by Olaifa and Erhun (1998). Similarly, maximum mean Developmental period of 52.66 days were recorded for storage duration of 100 days; however, it was statistically at par to 51.75, 51.25 days for storage interval of 80 and 60 days respectively, while lowest developmental period of 0 days was recorded at 40 days storage interval. These results are in line with Chinwada and Giga (1993). Significant differences were also observed for interactive effect of NLP and storage duration for developmental period indicated that with storage duration developmental period increased as the toxic effect of NLP reduced with a passage of time.

Table-1. Numbers of eggs laid and Developmental period as affected by applied treatments

Neem leaf powder (mg $100g^{-1}$)	No. of eggs laid	Developmental period (Days)
Control	138.24a	34.25d
0.5	117.82b	38.00c
1.0	102.18c	41.75b
1.5	88.75d	42.91a
$LSD_{(0.05)}$	6.67	0.937
Storage Duration (Days)		
40	101.20c	0.00b
60	109.05b	52.50a
80	120.69a	51.75a
100	116.05a	52.66a
$LSD_{(0.05)}$	6.67	0.937
NLP* Storage Duration		
Sig. level	NS	*(Fig 1)

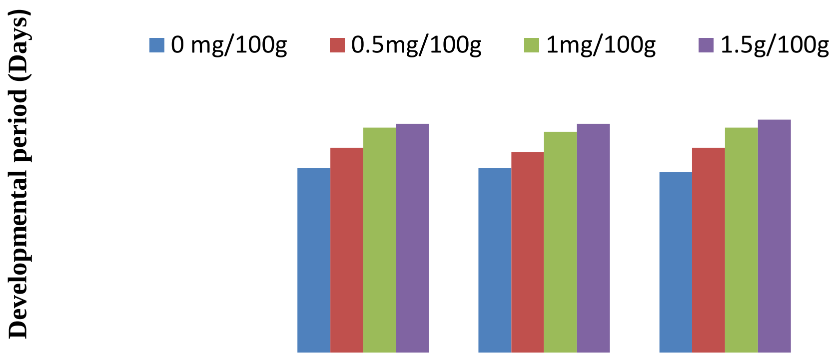

Figure 1. Developmental period as affected by interaction of neem leaf powder and storage duration

Total progeny

NLP and storage duration significantly affected the total progeny of pulse beetle in mungbean (Table-2). Maximum mean total progeny of 101.33 were recorded for NLP applied @ of 0 mg 100 g^{-1} grains followed by 87.83 for 0.5 mg 100 g^{-1} grains that was statistically higher than 71.83 observed for NLP applied @ 1 mg 100 g^{-1}seeds. While significantly minimum mean of 63.25 was observed for 1.5 mg NLP 100 g^{-1} seeds. These findings demonstrated that with the increase in NLP concentration, number of total progeny decreased. Similar results are reported by Bright *et al*. (2001) and Raja *et al*. (2001) who found that botanicals inhibited adult emergence in *C. maculates* in cowpea. They further stated that, when the eggs were laid on treated seeds, the toxic substance present in the extract may enter into the egg through chorion and suppressed their embryonic development which results in decreasing number of progeny. Likewise, maximum mean total progeny 86.58 were recorded for storage duration of 80 days; however, it was statically similar to 83.83 of 100 days. Storage duration of 60 days showed 80.33 total progeny that was significantly lower than that of 73.50 for 40days. It was also evident from result that with the increase in storage duration total progeny increased due to F2 generation that emerged from egg laid by previous generation. Non-significant differences were observed for interactive effect of NLP and storage duration for total progeny.

Adult mortality

The effects of NLP and storage duration were significant for adult mortality of plus beetle in mungbean (Table-2). Maximum mean mortality of 41.55% were recorded for NLP applied @ of 1.5 mg 100 g^{-1} grains followed by 36.24% where NLP was used @ 1 mg 100 g^{-1}

grains that was statistically higher than 31.40% observed for NLP applied @ 0.5 mg 100 g^{-1} seeds, while significantly lower than control. Minimum mean of 25.65% was observed for control. These results are in line with Doharey and Singh (1989) and Sharma *et al*. (1980) who reported that the presence of azadirachtin compound that works as anti feedent deterrent and also as an inhibitor of ecdysis and growth contributes to adult mortality. Maximum adult mortality 53.49% was recorded for storage duration of 100 days; that was statistically higher than 38.23% of 80 days. Storage duration of 60 days showed 31.41% adult mortality that was significantly higher than that of 11.71%adult mortality observed at 40 days of storage duration. It was also noted that adult mortality increased with increase in storage duration. It might be due to F2 generation that emerged from egg laid by previous generation and due to the presence of lethal/ toxic effect of NLP which results in further mortality of the adults as reported by Chandrakala *et al.* (2013) who stated that with increase in duration adult mortality also increased.

Table-2. Total progeny and adult mortality

Neem leaf powder (mg $100g^{-1}$)	Total progeny	Adult mortality
Control	101.33a	25.65d
0.5	87.83b	34.40c
1.0	71.83c	36.24b
1.5	63.25d	41.55a
$LSD_{(0.05)}$	6.54	3.51
Storage Duration (Days)		
40	73.50b	11.71d
60	80.33a	31.41c
80	86.58a	38.23b
100	83.83a	53.49a
$LSD_{(0.05)}$	6.54	3.51
NLP * Storage Duration		
Sig. level	NS	NS

Grain damage %

Neem leaf powder and storage duration significantly affected % grain damaged by plus beetle in mungbean (Table-3). Maximum mean grain damage of 24.53% was recorded for control and minimum mean grain damage of 13.55% was observed for 1.5 mg NLP 100 g^{-1} grains. It was evident from result that with increase in NLP concentration % grain damage decreased. Similar results were also obtained by Sharma *et al*. (1980) who reported that with application of NLP grain damage was reduced by 23%. Maximum mean %grain damage 24.45 was recorded for storage duration of 100 days; however, it was statically at par with 20.06 of 80 days. Storage duration of 60 days showed 17.04% grain damage that was significantly lower than that of 13.55% recorded for 40 days. These results showed that with the increase storage duration %grain damage increased. It might be due to F2 generation that emerged from egg laid by previous generation and due reduction of lethal/ toxic effect of NLP with passage of time, same results have been reported by (Chandrakala et al., 2013) who stated that with increase in storage duration %grain damage increases.

Weight loss%

Neem leaf powder and storage duration significantly affected % weight loss in mungbean (Table-3). Maximum mean weight loss % of 30.85 were recorded for control where no NLP was applied followed by 20.97 % of 0.5 mg NLP 100 g^{-1} grains that was statistically higher than 17.50% observed for NLP applied @ 1 mg 100 g^{-1} seeds. Minimum mean % weight loss of 14.46 % was observed for 1.5 mg NLP 100 g^{-1}grains. These results declared that with increase in NLP concentration, weight% loss decreased. These results agree with Sharma et al., (1980). Maximum mean % weight loss30.65 was recorded for storage duration of 100 days; however, it was statistically at par to 20.92% of 80 days. Storage duration of 60 days showed 17.94% weight loss that was significantly higher than that of 14.27% weight loss observed for 40days of storage duration. It was also evident from result that with the increase storage duration weight loss % increased. It might be due to F2 generation that emerged from egg laid by previous generation and due to the decrease of lethal/ toxic effect of NLP with passage of time it may slows down its effect as cited by Sharma *et al*. (1980). These result demonstrated that generally with increasing rate of NLP the egg laying and adult emergence were significantly reduced which contributed to less weight loss. Our results are in line with findings of Chandrakala *et al*. (2013) too.

CONCLUSION

It is concluded from the results that with increase in neem leaf powder concentration number of egg laid, developmental period, total progeny, %grain damage and % weight loss decreased while adult mortality increased. Similarly, with the increases in storage duration number of egg laid, developmental period, adult mortality, total progeny, %grain damage and % weight loss increased. Neem leaf powder applied at the rate of 1.5 mg/100 g grains performed better as compared to control and other concentrations.

Table-3. Grain damage and weight loss affected by applied treatments

Neem leaf powder (mg $100g^{-1}$)	Grain damage (%)	Weight loss (%)
Control	24.53a	30.85a
0.5	20.24b	20.97b
1.0	16.77c	17.50c
1.5	13.55d	14.46d
$LSD_{(0.05)}$	1.98	1.99
Storage Duration (Days)		
40	13.55d	14.27d
60	17.04c	17.94c
80	20.06b	20.92b
100	24.45a	30.65a
$LSD_{(0.05)}$	1.98	1.99
NLP * Storage Duration		
Sig. level	NS	NS

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