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Effectiveness of Nimbecidine for the Control of Pulse Beetle, *Callosobruchus Chinensis* on Stored Pulses

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Abstract:

Use of plant to control pests has been a traditional method in world over and this has received interest in recent times. Botanical such as neem and its products have potential as insect control agents and recent studies have shown that such compounds may be effective against stored product insects. Neem affected feeding, growth and development of C. chinensis.

The neem formulation Nimbecidine (0.03% azadirachtin) was assayed to find out its effectiveness for the control of pulse beetle, Callosobruchus chinensis ,on stored moong (Phaseolus aureus) .it was observed that Nimbecidine adversely affected growth and development of this insect producing growth retardation and sterility at 1% and 2% Nimbecidine and demorphogenesis in the larvae,pupae and adults at 3%.Besides acting as an ovipositional deterrent, Nimbecidine was ovicidal at 4%.Adult mortality was also found to be dose dependent; at 5% Nimbecidine 94% of the adults died within 24h of treatment and Nimbecidine was found to be effective up to 90 days. Nimbecidine was found not to affect the seed viability as the germination percentages were being within the range of 74% to80%. This showed the potentiality of Nimbecidine as a biopesticide against Callosobruchus chinensis.

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Key words: Botanicals, Nimbecidine,Malathion, *Callosobruchus chinensis*, *Phaseolus aureus*

Introduction

Pulses in storage are damaged by many pests mainly the bruchids including *Callosobruchus chinensis*. Control of pest is difficult in the storage of grains.

Nearly 80% of stored seeds are damaged by many pests including the bruchids. Damage due to the stored grain insect pests in case of pulse is relatively more (5%) as compared with the major food grain like rice (2%), wheat (2.5%) and maize (3.5%) (Jeswani and Baldev, 1990). Applications of synthetic persistent insecticides for protecting grains are broadly discouraged. Use of plant parts including the Indian neem tree (Azadirachta *indica* A. Jass) (Meliaceae) leaves and seeds as grain protectant has been practiced by the farming community since time immemorial (Kalita et.al 2002). Neem products have potential as insect control agents and recent studies have shown that such compounds may be effective against stored product insects. Neem products contain azadirachtin which interferes with larval growth and development of an insect by causing morphological and physiological abnormalities. Growth retardation, molting inhibition induction of malformation are some of such abnormalities which are reported by Schmutterer and Ascher (1986). The neem products were found effective in reduction of percentage of adult emergence and lengthening the developmental period. The adverse effects of neem products on oviposition and hatching of eggs had so far been reported by several workers (Lakwah et al 1994; Rouf et al 1996, Chiranjeevi and Sudhakar, 1996).The adverse effect on adult emergence after application of neem seed poweder and neem oil against *Callosobruchus* are also reported by Suksomchit(1988), Naik and Dumbre (1984) and Ahmed et al(1993). Antifeedent properties of NSO may directly influence the nutrition, growth and development and ultimately survival of an insect. It acted as an antiovipositional and ovicidal toxicant against *Callosobruchus* sp. (Yadav, 1985). In 1990 Schmutterer reviewed properties and potential of neem pesticides in which he mentioned that neem products showed a considerable potential in the control of store insects like Sitophilus, Tribolium and most importantly pulse beetles, bruchids. There are many neem oil based formulations commercially available in the market. Considering the efficacy of various neem extracts on the pests, some workers used commercially available neem formulations on different pests including pulse beetle,*C.chinensis*(Lakwah et al.,1994; Vyas et al.,1999; Umrao and Verma 2002b; Kumar et al.,2003; Kashlan,2004;) So such works were initiated to evaluate the effectiveness of a neem formulation Nimbecidine for the control of *Callosobruchus chinensis* on stored pulses.

Materials and Methods

Insect Culture:

C.chinensis was reared on moong (*Phaseolus aureus*) at room temperature (16-31^{0}c) and relative humidity (60-94%).The cultures were maintained in transparent plastic jars (11.5cmX7.5cm) one fourth filled with seeds.

Treatment of moong seeds by different concentrations of Nimbecidine:

Nimbecidine is a commercial neem formulation having 0.03% EC Azadirachtin in it. It is obtained from T.Stanes & Company Ltd. 8/23-24 Race Course Road Coimbatore-641018.

Different concentrations were prepared by taking 1ml, 2ml, 3ml, 4ml and 5ml of Nimbecidine in 100ml of distilled water. For each treatment 50 healthy seeds were treated with 0.2ml of 1%, 2%, 3%, 4%, and 5% Nimbecidine, Malathion (0.05%) and an untreated control. The seeds were kept in small glass vial (3.5cmx7cm). After treatment five pairs of newly emerged beetles were released inside each vial, mouth of which were covered with muslin cloth for proper aeration.

Data Collection:

The numbers of eggs laid in each treatment were counted 24hrs after release of the females. Seeds were observed daily and hatching percentage was also recorded. Adult emerged after cutting a circular hole in the seed coat. The number of adults emerged were counted and the developmental period was also observed. The periods taken from the day of releasing insects up to the day of first emergence and again from the 10^{th} day of releasing insects up to the last day of emergence were recorded for each vial. The average of these two periods was taken as average developmental period.

100g seeds were treated with Nimbecidine at rates from 0.4%(1.2ppm) to 5%(15ppm) .The seeds were kept in glass jars and allowed to be infested by *C. chinensis*. in natural preference inside a closed chamber. Data were collected for percent infestation, after 30 days, 60 days and 90 days of storage. Average data of 5 samples of seeds with emergence holes were taken to calculate the percent damaged seeds.

One hundred seeds were treated with Nimbecidine at rates 0.5% (1.5ppm) to 5 %(15ppm), 0.05% malathion on which 10 pairs of < 4h old adults of *C. chinensis* were released. Data on mortality of these beetles were scored daily until the death of the adults.

To find out the effect of Nimbecidine on seed viability germination tests were carried out. Fifty number of seeds were treated with different concentrations of Nimbecidine and were kept on moistened filter papers on petridishes; the numbers of seed germinated in each treatment after 48 hours were counted and the length of growing shoots were measured. Germination test was done similarly for the untreated control also.

Results and Discussion

Nimbecidine reduced the rate of egg laying and percentage of hatching at all the doses tested (Table 1).The effect was maximum at 5% Nimbecidine which was similar to that of 0.05% malathion. Similarly emergence was equally affected, with the concentration, i.e. higher the concentration more the effect.

Table 1: Effect of Nimbecidine on development of *Callosobruchus chinensis*

Treatments	Mean eggs± SD/0+/day	Mean hatching±SD(%)	Mean emergence ±SD (%)	Mean developmental period ± SD
1% Nimbecidine	6.4±1.5	41.6±10.6 (40.05)	36.6±18.5(34.09)	28.4±0.49
2% Nimbecidine	6±1.9	38±6.3(38.40)	36.6 ±18.5(34.09)	29.6±0.49
3%Nimbecidine	4±1.4	26.3±7.6(28.02)	0(0.41)	0
4% Nimbecidine	2.6±1	0	0	0
5% Nimbecidine	0	0	0	0
Malathion(0.05%)	1.8±0.75	0	0	0
Control	32.2±6.3	90.6±3.8(72.69)	86.9±5.2(69.20)	23±1.4
S..Ed.	1.88	5.84	8.79	0.42
$CD_{0.05}$	3.84	12.37	18.63	0.86

The figures in the parenthesis are Angular Transformation Value

Table 2: Effect of Nimbecidine on adult morphological characters of *Callosobruchus chinensis*.

		Body length (mean±SD)	Body width (mean±SD)	Antennal length (mean±SD)	Elytral length (mean±SD)	Elytral width (mean±SD)
Male	Control	2.70±0.40	2.20±0.40	2.30±0.08	1.80±0.06	0.90±0.06
	Treated	1.50±0.32	0.82±0.70	0.98±0.32	0.80±0.14	0.37±0.70
	t-value	9.80	12.24	9.57	12.56	13.02
Female	Control	3.70±0.40	2.70±0.40	2.10±0.08	1.90±0.06	1.00±0.06
	Treated	1.70±0.37	0.90±0.20	0.90±0.43	0.82±0.18	0.40±0.10
	t-value	25.25	15.23	6.46	13.90	17.50

Table 3. Effect of Nimbecidine as grain protectant against *Callosobruchus chinensis* over certain periods.

Treatments	Mean ± SD% seeds damaged		
	30 days	60 days	90 days
Control	59.000±3.162 (50.194)[a]	88.200±0.447 (69.911)[a]	100.000±0.000 (90.000)[a]
0.4% Nimbecidine	33.200±0.447	40.000±1.581	57.400±0.548

	(34.763)[b]	(38.974)[b]	(50.183)[b]
0.50%Nimbecidine	29.400±1.673 (32.828)[c]	36.200±1.095 (36.988)[c]	47.000±2.121 (43.279)[c]
1% Nimbecidine	20.400±1.517 (26.839)[c]	30.800±1.086 (33.707)[d]	42.200±0.447 (40.512)[d]
2% Nimbecidine	18.200±0.837 (25.248)[d]	29.800±1.304 (33.082)[d]	40.600±0.894 (39.581)[e]
3% Nimbecidine	0	0	0
4% Nimbecidine	0	0	0
5% Nimbecidine	0	0	0
Malathion (0.05%)	0	0	0
S.Ed.	0.698	0.451	0.393
CD(5%)	1.455	0.941	0.821

The figure in the parenthesis are A.T. Value.
Means followed by the same letters are not significantly different from each other (P=0.05), n=50

Table 4. Effect of Nimbecidine on (mean±SD) mortality of *Callosobruchus chinensis.*

Treatments	Adults died within 24 h	Mortality (%)
0.5% Nimbecidine	0.000 ± 0.000	0.000±0.000 (0.025)
1% Nimbecidine	1.800 ± 0.837	18.000[d] ± 8.367 (24.642)
2% Nimbecidine	2.600 ± 0.548	26.000[d] ± 5.477 (30.553)
3% Nimbecidine	4.200 ± 0.837	42.000[c] ± 8.367 (40.335)
4% Nimbecidine	6.400 ± 0.894	64.000[bc] ± 8.944 (53.227)
5% Nimbecidine	9.400 ± 0.890	94.000[b] ± 8.944 (86.985)
Malathion (0.05%)	10.000 ± 0.000	100.000[a] ± 0.000 (99.975)
Control	0.000±0.000	0.000±0.000 (0.025)
S. ed		4.634
CD 5%		9.458

The figure in the parentheses are A.T. value.
Means followed by the same letters are not significantly different from each other (P=0.05), n=50

Table 5. Effect of Nimbecidine on germination of moong seeds.

Treatments	Number of seeds treated	Mean number of seeds germinated within 48 h	Germination Percentage (%)	Highest shoot length within 48 h (mm)
Control	50	40.000 ± 1.000	80.000 ± 2.000	5.500[a] ± 0.071
0.5% Nimbecidine	50	40.000 ± 1.581	80.000 ± 3.16	5.400[a] ± 0.158
1% Nimbecidine	50	39.000 ± 0.707	78.000 ± 1.414	4.800[b] ± 0.071
2% Nimbecidine	50	39.000 ± 2.000	78.000 ± 4.000	4.800[b] ± 0.100

3% Nimbecidine	50	38.000 ± 0.707	76.000 ± 1.414	4.600[c] ± 0.061
4% Nimbecidine	50	38.000 ± 1.581	76.000 ± 3.162	4.400[d] ± 0.071
5% Nimbecidine	50	37.000 ± 1.225	74.000 ± 2.449	4.200[e] ± 0.154
Malathion (0.05%)	50	36.400 ± 4.159	72.800 ± 8.319	3.500[f] ± 0.158
S. ed				0.072
CD 5%		NS	NS	0.146

Means followed by the same letters are not significantly different from each other (P=0.05), n=50

Nimbecidine affected the size of the emerged adults (Fig.1). Adults emerged out of the treated seeds with 1% and 2% Nimbecidine were the smallest male being 1.5 mm long and 0.8 mm wide while female being 1.7mm long and 0.9 mm wide in comparison to 3 mm length and 2.5 mm width in normal male and 4 mm length and 3 mm width in normal female. These smaller 1% and 2% Nimbecidine treated emerged females were found sterile.

Nimbecidine affected the size of the elytra and antennae also (Table 2). Above effects were seen in the control if the food was not adequate. The effect on the size of the adults may be attributed to the inadequacy of suitable food for the larvae, which resulted growth retardation in these individuals.

Emergence was blocked in 3% Nimbecidine treated seeds. Dead larvae, adult and their intermediate stages inside these seeds showed morphological abnormalities (Fig2). Morphological abnormalities occurred in the size of the adults, size of the elytra, folding mechanism in the second pair of wings, size of the abdomen, size of the antennae and mouth parts. These morphological abnormalities were found to be almost similar to those described on *Callosobruchus maculatus* by Rup and Chopra,1984

An adverse effect of developmental abnormalities in almost all insect orders was reported to be caused by Azadirachtin-A isomer (Zanno et al.1995).Nimbecidine affected the developmental period. Lower doses of 1% and 2% Nimbecidine increased the developmental period 27 to 32 days compared to 23 days in control.

Percentage of damaged seeds differed significantly amongst the treatments on 30th, 60th, and 90th day of storage as 33.2,40 and 57.4 percent, respectively (Table 3). Similarly, adult mortality was also significantly different among the treatments, the highest being in case of malathion (100%) followed by 5% Nimbecidine (94%), however, no such effect was observed in case of 0.5% Nimbecidine (Table 4). Sangwan et al. (2005) found neem oil effective upto 35 days of storage on the basis of adult mortality (64.33%) of *C. maculatus*.

Germination test conducted revealed that the germination percentage did not differ significantly among those treatments which ranged between 74 to 80 percent, however, shoot length was significantly different amongst the treatments(Table 5). Kashlan(2004) reported that the germination of the Neemazol treated cow pea seeds was almost equal to the control.

In summary, Nimbecidine affected growth and development of *C. chinensis.* From the Germination test of the treated seeds, Nimbecidine was found not to affect the seed viability. This indicated the effectiveness of Nimbecidine for the control of pulse beetle, *Callosobruchus chinensis* on stored pulses.

Body size

A **B** **C** **D**

A. Dorsal view of normal male (Actual body length: 2.70±0.40mm)
B. Dorsal view of normal female (Actual body length: 3.70±0.40mm)
C. Retarded male (Actual body length: 1.50±0.32mm)
D. Retarded female (Actual body length:1.70±0.37)

Size of antennae

A B C D

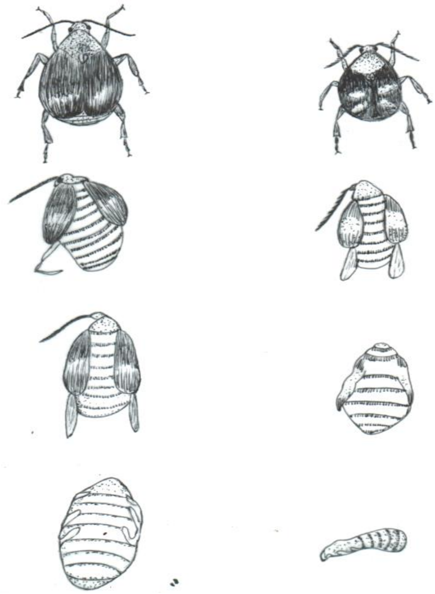
A. Normal male antennae
B. Normal female antennae
C. Antennae of a treated male
D. Antennae of a treated female

Size of elytra

A B C D

A. Normal male elytra
B. Normal female elytra
C. Elytra of a treated male
D. Elytra of a treated female

Fig.1. Growth retardation induced by 2% Nimbecidine in *C.chinensis*



I. Dorsal view of normal female (actual body length: 3.70±0.40mm)
II.Dorsal view of normal male (actual body length: 2.70±0.40 mm)
III. Dorsal view of morphologically deformed female showing diverging elytra not meeting on dorsal side.
IV. Dorsal view of male showing short elytra not cover the abdomen and second pair wings unfolded.
V. Dorsal view of female with both pairs of wings deformed.
VI. Dorsal view of adultoid with incomplete development of organs.
VII. Ventral view of adultoid with poorly developed head and thoracic appendages.
VIII .Larval-pupal intermediate

Fig.2. Morphological aberrations induced by 3%Nimbecidine in C.chinensis

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