TITLE: **PROTECTION OF THE POST-HARVEST PULSE GRAINS WITH NAPHTHALENE BALLS AGAINST THE ATTACK OF THE PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* (L.), IN THE NONREFRIGERATED HOMESTORES.**

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Abstract- Naphthalene ball or moth ball, an almost non-toxic chemical compound, was tested for the protection of the pulse beetle, *Callosobruchus chinensis* (L.), in stored chickpea (*Cicer arietinum*), mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*), and lentil (*Lens esculentus*). The adult beetles did not emerge from the freshly laid eggs after a maximum period of 7-day exposure with naphthalene balls in closed containers having either chickpea, mungbean, lentil, or cowpea. A further study indicated that chickpea grains with freshly laid eggs of the beetle showed no adult beetle emergence even after 24 hours of exposure with naphthalene. The effective exposure period of naphthalene varied for hidden larval or pupal mortality as well as in different pulses. The adult emergence of the pest from the exposed cowpea, chickpea, mungbean, or lentil with hidden larvae or pupae were almost nil after a maximum exposure period of 144 hours in chickpea as compared with the unexposed grains. One hundred percent adults died after 24-hours exposure with naphthalene balls in closed containers having chickpea. Retrieved grains immediately after 60-day exposure with naphthalene seemed to be non-toxic as there were no significant differences ($P>0.05$) in the number of eggs laid and the adults emerged in grains so retrieved and untreated grains. There were no significant differences ($P>0.05$) in the percent of germination of pulse grains treated for up to 60 days with naphthalene and the untreated grains. One kg of pulse beetle infested chickpea grains exposed for 10-15 days with four naphthalene balls gave complete control of the pulse beetle and the fungal growth for a minimum period of seven months in any types of sealed containers.

INTRODUCTION

The pulse beetle, *Callosbruchus chinensis* (L.), causes a minimum of 30% losses in the storehouses every year in Bangladesh except blackgram (*Vigna mungo*) (Islam, 1986). The principal method of controlling the pest in large storehouses is fumigation with toxic chemicals but in the nonrefrigerated homestores, farmers hardly take any control measure due to nonavailability of any cheap or easy-to-use technology except sundrying of pulse grains before storage. However, among the non-toxic methods of controlling the pest, there are a few technologies such as the use of different oils of castor, mustard, coconut, sesame, etc., (Verma and Pandey, 1978; Mummigatti and Ragunathan, 1977; Naik and Dumbre, 1985), camphor (Abivardi, 1977), hot water and dry sand (Rahman, 1990) treatments against the attack of the pulse beetle, has not been accepted widely by the farmers. In this study, we have diverted our efforts for finding an effective method with relatively cheaper and readily available material for the control of the pulse beetle in the homestores.

Naphthalene is almost non-toxic and it is being used mostly as a fumigant in houses and and as an animal repellent (George, 1980). Since it is safe, readily available every where, very cheap, and requiring only a small quantity to saturate the air (Shepard, 1951), a study was undertaken to determine the lethal effects of naphthalene balls on different developmental stages of the pulse beetle (*C. chinensis*) in pulse grains stored in different container types.

MATERIALS AND METHODS

All the experiments were conducted in the laboratory of the Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, during 1989-90 using the laboratory strain of the pulse beetle, *Callosobruchus chinensis* (L.) at ambient temperature ($29.5 \pm 1.8^{0}C$) and r.h. ($77.8 \pm 11.6\%$).

EFFECT OF NAPHTHALENE ON EGG MORTALITY

Fifty pairs of newly emerged pulse beetle adults were released in a jelly jar (11cm x 6cm dia) containing two hundred healthy grains of lentil for egg laying for two days. Twenty-five randomly selected lentil grains with fresh eggs were introduced in each of the three glass vials (13cm x 2.5cm). One naphthalene ball (2.0 g) was put for seven days in each vial, and all the vials were plugged with cotton wool. Similarly, three vials with eggs on grains from the same jar were kept without any naphthalene ball as the untreated check (control). The number of larvae hatched per grain (by observing the egg shell full of white powdery excreta with a dissecting microscope) after seven days and at emergence the number of adults emerged both in the treated and in the control vials were recorded. Similar methods were followed for egg mortality study in chickpea, mungbean, and cowpea.

For studying the critical exposure period of pulse grain with naphthalene for egg mortality, two naphthalene balls were introduced in 300 grains of chickpea having freshly laid eggs in a closed jelly jar. At every 24h exposure interval up to 96h, 10 randomly selected grains were introduced in each of the three closed glass vials and were kept for adult emergence. For the untreated check (0h exposure), pulse grains with eggs were introduced in glass vials immediately before the naphthalene application. Data were recorded on the mean number of larvae hatched and adults emerged per 10 grains for all exposure periods.

EFFECT OF NAPHTHALENE ON LARVAL, PUPAL, AND ADULT MORTALITY

Larval mortality: Eightly pairs of the pulse beetle adults were released for egg laying in a closed jelly jar having one thousand healthy chickpea grains. After five days, the adults were removed. On the 10th day after the start of egg laying (considered as larval stage which was confirmed by splitting the grains for larvae) two naphthalene balls were introduced into the jar. Three samples of 25 grains each were drawn after every 24h of the naphthalene treatment and were put to the saparate glass vials with cotton plugs. For the untreated check three glass vials, each having 25 grains with eggs from the same batch of grains were isolated just before the naphthalene application. Data on the number of adults emerged in both the treated and the untreated vials were recorded for all the expsoure periods until last adults were found to emerge. Similar methods were applied for studying the larval mortality in lentil, mungbean, and cowpea.

Pupal mortality: Exactly identical methods to that of the larval mortality were followed except the naphthalene application was made on the 16th day after egg laying (considered as pupal stage which was confirmed by splitting the grains for pupae).

Adult mortality: Twenty-five adults of the pulse beetle were released in each of the two closed jelly jars having 100 healthy chickpea grains. Five minutes later, one naphthalene ball was put in one jar and the other jar was kept as control. The number of adult died were recorded at 24h exposure intervals.

RESIDUAL TOXICITY STUDY OF NAPHTHALENE TREATMENT ON PULSE GRAINS

Two naphthalene balls were added to 150 g of chickpea in a closed jelly jar. Immediately after 60 days of continuous exposure to naphthalene, 25 grains were randomly introduced in each of the three glass vials. In the untreated vials, chickpea grains without naphthalene treatment were kept. In each vial, 10 pairs of the adult beetles were released for egg laying. Data on the number of eggs laid, the number of larvae hatched, and the number of adults emerged were recorded. Similar studies were also done for mungbean.

EFFECT OF NAPHTHALENE ON GERMINATION

Five hundred grains of mungbean were introduced in each of the two closed jelly jars. In one jar, two naphthalene balls were added and the other jar was kept as untreated check. On the 45th day and the 60th day, 25 grains were put in each of the three petri dishes (9cm dia) from both the treated and the untreated jars for germination tests. In this way germination tests for naphthalene treated and untreated grains of chickpea, mungbean, lentil, and cowpea were studied.

EFFECT OF NAPHTHALENE TREATMENT IN DIFFERENT CONTAINER TYPES

Polythene bags (20cm x 15cm), jelly jars with plastic lids (11cm x 6cm), earthen jars (13cm x 11cm dia) with earthen lids, and 'DANO' milk powder containers (12cm x 10cm dia) with lids were used for storing the chickpea grains. In each container, 150 g of healthy and 200 infested chickpea grains were introduced. In one set, two naphthalene balls were put in each container and the other set received no naphthalene balls to serve as untreated check. After 30 days, naphthalene balls were removed from the containers and were kept closed again. All the treatments were replicated six times. After 60 days, data on the percentage of bored grains and the grains with eggs were recorded.

EFFICACY OF DIFFERENT DOSAGES OF NAPHTHALENE BALLS

Four dosages (one, two, three, and four balls) of naphthalene balls (2 g each) were tested for their efficacy in controlling the pulse beetle. For each treatment, one kg of the healthy chickpea grains was taken in an earthen jar (20cm x 17cm dia) and 200 pulse beetle infested grains of chickpea were added. After the treatment application, all the jars, including the untreated check were sealed with mud. After 15 days, naphthalene balls were removed and the jars were sealed again. Each treatment was replicated six times. After 60, 90, and 210 days of storage, percentage of bored grains and the grains with eggs were recorded.

RESULTS

The pulse beetle larvae did not hatch and the adults did not emerge from the pulse grains (chickpea, lentil, cowpea, and mungbean) having eggs exposed to naphthalene for 7 days as compared with the unexposed grains (Table I).

Table I. Effect of 7d naphthalene-exposure on egg mortality of the pulse beetle in different pulses.

Treatment	Chickpea		Mungbean		Cowpea		Lentil	
	MEH/G	MAE	MEH/G	MAE	MEH/G	MAE	MEH/G	MAE
One naphthalene	0.03	0	0.03	0	0.03	0	0	0
Control	2.15	31.67	1.31	22.67	4.75	102.0	2.84	40.67

MEH/G=Mean number of eggs hatched per grain; MAE= Mean number of adults emerged. Mean of three replicates; in each replicate, 25 grains were used.

A further study indicated that there was almost no adult emergence from chichpea grains with freshly laid eggs when exposed to naphthalene balls for even 24h in a closed container (Table II).

Table II. Effect of exposure periods of two naphthalene balls on the egg mortality in chickpea.

Exposure (h)	Mean number of Larvae hatched		Adults emerged
0(Control)	58.00	a	46.00
24	2.33	b	0.33
48	2.67	b	0
72	0	b	0
96	0	b	0

Mean of 3 replicates; 10 grains were used in each replicate.
Mean followed by common letter(s) do not differ significantly (P>0.05) by Duncan's Multiple Range Test (DMRT).

There was no adult emergence from the infested pulse grains with hidden larvae after 24, 48, 120, and 144 hours of exposure with naphthalene balls for mungbean, cowpea, chickpea, and lentil, respectively (Table III)in closed glass jars. Whereas no adult emergence was observed from infested cowpea, lentil, mungbean, and chickpea grains with hidden pupae after 120, 144, 144, and 192 hours of exposure with naphthalene balls in closed glass jars, respectively (Table IV). Even a 24 h exposure of 25 adult pulse beetle with a single naphthalene ball in a closed glass jar gave 100% mortality of the beetle (Table V).

Table III. Effect of two naphthalene balls at different exposure periods on pulse beetle larval mortality in closed glass jars in different pulses.

Exposure periods(h)	Mean number of adults emerged from Cowpea	Chickpea		Lentil		Mungbean
0h(Check)	5.33	39.67	a	25.33	a	13.67
24h	1.00	16.67	b	6.33	b	0
48h	0	11.67	c	6.00	b	0
72h	0	6.33	d	4.67	b	0
96h	0	5.00	d	1.67	b	0
120h	0	0	e	1.33	c	0
144h	0	0	e	0	c	0

Mean of three replicates; in each replicate, 25 grains were used. Means followed by common letter(s) in the column do not differ significantly (P>0.05) by DMRT.

Table IV. Effect of two naphthalene balls at different exposure periods on the pulse beetle pupal mortality in different pulses in closed glass jars.

Exposure periods(h)	Mean number of adults emerged from			
	Chickpea	Cowpea	Lentil	Mungbean
0h(Check)	119.00 a	36.00 a	28.67 a	12.33 a
24h	92.67 b	47.33 a	23.00 b	10.67 b
48h	65.00 c	42.67 a	19.00 b	7.00 c
72h	74.67 c	24.00 b	12.00 c	5.33 d
96h	29.67 d	3.33 c	2.67 d	2.00 e
120h	9.00 e	0 c	1.33 d	2.00 e
144h	9.33 e	0 c	0 d	0 f
168h	3.33 e	0 c	0 d	0 f
192h	0 e	0 c	0 d	0 f

Mean of three replicates; in each replicate, 25 grains were used; Mean followed by common letter(s) in the column do not differ significantly (P>0.05) by DMRT.

Table V. Effect of one naphthalene ball on the adult pulse beetle mortality in a closed glass jar.

Treatment	Mean adults died in 24h	Mean eggs laid before adults died	Mean adults emerged
Naphthalene	25.00	2.33	0
Untreated	0	23.00	22.33

Mean of 3 replicates; N=25.

In toxicity studies, the mean number of eggs laid, eggs hatched and adults emerged did not differ significantly for 60d naphthalene-exposed and unexposed pulse grains (Table VI). Various durations of naphthalene exposure to pulse grains up to 60d showed no effect on the germination of the pulse grains (Table VII).

Table VI.Mean number of egg laid, eggs hatched and adult emerged from retrieved pulses exposed to two naphthalene balls in closed glass jars for 60 days.

Treatment	Chickpea			Mungbean		
	MEL	MEH	MAE	MEL	MEH	MAE
60d exposed	232.0	217.33	151.67	161.0	114.33	62.67
Unexposed	257.33	228.0	166.33	157.33	105.0	54.67
t-value	1.44	0.39	1.02	0.14	0.31	0.89

MEL=Mean number of eggs laid; MEH=Mean number of eggs hatched; MAE=Mean number of adults emerged; Mean of three replicates; in each replicate, 25 grains were used.

Table VII. Effect of exposure periods with two naphthalene balls on the germination of lentil, cowpea, mungbean, and chickpea.

Pulse grains	Mean percent of germination at exposure periods 45d		60d	
	Exposed	Unexposed	Exposed	Unexposed
Lentil	98.68	98.68	100.00	90.68
Cowpea	77.32	81.32	80.00	81.32
Mungbean	85.32	90.68	93.32	84.00
Chickpea	85.32	89.32	92.0	90.60

Mean of 3 replicates; in each replicate, 25 grains were used.

Exposure of pulse grains with naphthalene ball in different container types showed excellent results except for polythene bags where the percentage of bored grains and the grains with eggs in 30d naphthalene-exposed chickpea were significantly higher ($P<0.05$) than the rest of the container types but lower than the control treatments (Table IX).

Table IX. Effect of 30 days exposure periods with two naphthalene balls in different container types on the percentage of pulse beetle infestations in chickpea.

Container types	Mean percentage of bored grains		grains with eggs	
	Treated	Control	Treated	Control
Polythene bag.	9.00	100	57.59	100
glass jar with nonperforated lid.	0	100	0	100
Glass jar with perforated lid.	0	100	0	100
Earthen jar with loose lid.	0	100	0	100
Earthe jar with sealed lid.	0	100	0	100
'DANO' container with sealed lid.	0	100	0	100

Mean of six replicates; Initial infestation: 200 pulse beetle infested grains in 150 g of chickpea per treatment per replication.

The lowest significant ($P<0.05$) mean number of bored grains and the grains with eggs were obtained with four naphthalene balls for 210 days of storage compared with the untreated grains and the grains treated with one, two, and three naphthalene balls (Table VIII).

Table VIII. Efficacy of different dosages of naphthalene balls exposed for 15 days in closed earthen jar on the infestations of chickpea.

Naphthalene balls	60 days		90 days		210 days	
	MBG	MEG	MBG	MEG	MBG	MEG
Control	114.17b	85.83a	200.0a	200.0a	200.0a	200.0a
One	123.50a	76.50a	200.0a	200.0a	200.0a	200.0a
Two	8.00c	85.33a	44.0b	76.17b	200.0a	200.0a
Three	0.0 c	15.17b	1.83c	20.33c	1.5b	41.0b
Four	0.0 c	1.67b	0.0 c	0.0 c	0.0c	0.0c

Mean number of bored grains; MEG: Mean number of grains with eggs. Mean of six replicates; sample size was 200 grains per treatment per replicate; Means followed by common letter(s) in the column do not differ significantly (P>0.05) by DMRT.

DISCUSSION AND CONCLUSIONS

A 24 h exposure period with naphthalene balls in closed containers was sufficent to give 100% mortality of eggs and adults of the pulse beetle in stored pulses. For infested pulse grains having hidden larvae and pupae of the pulse beetle, a maximum exposure period with naphthalene balls in closed containers was 144 h for complete eradication of the developmental stages of the beetle, except for the pupal stage in chickpea where an exposure period of 196 h was needed. Herrick and Griswold (1933) and George (1978) reported that naphthalene flakes were toxic to eggs and larvae of the webbing clothes moth, *Tineola bisselliella* (Hum.), under prolonged confinement in a tight container. Lehman (1933) reported that naphthalene gave 50% kill of the confused flour beetle, *T. confusum* Duv., with an application rate of 1.1 g/litre. Naphthalene flakes and balls are also used for the control of insect pests damaging insect specimens in museums and entomological laboratories (Shepard, 1951).

The present study also showed that chickpea grains, with sources of pulse beetle infestation, could be kept free from any sign of the pulse beetle infestation and mould development for a prolonged period of 210 days by keeping the grains in a closed container with naphthalene treatment for the initial 15 days only at the rate of four balls per kg of pulse grains. Pulse grains treated with naphthalene balls for up to 60 days did not show any adverse effect on the germination and also did not show any toxic effect to adult beetles as evidenced from the residual toxicity studies.

Experimental data of the present study have provided basic importance of the feasibility of using naphthalene balls in the control of the pulse beetle in stored pulses under small farmers' storage conditions in developing countries. Further studies are necessary with large quantities of different pulse grains and storage container types for verification and standardization of naphthalene balls as an effective control agent of the pulse beetles in stored pulses.

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PROTECTION DES GRAINES DE LEGUMINEUSES APRES RECOLTE PAR LES BOULES DE NAPHTALINE CONTRE LES ATTAQUES DE LA BRUCHE, *CALLOSOBRUCHUS CHINENSIS* (L) (*COLEOPTERA : BRUCHIDAE*) DANS LES CELLIERS DOMESTIQUES NON REFRIGERES

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Résumé

Les boules de naphtaline, ou boules à mites, produit chimique presque exempt de toxicité, ont été étudiées quant à leur effet protecteur contre la bruche chinoise, *Callosobruchus chimensis* (L.) sur le pois chiche (*Cicer arietinum*), le haricot mungo (*Phaseolus mungo*), le niébé (*Vigna sinensis*) et la lentille (*Lens esculentus*). Les coléoptères adultes n'ont pas émergé des oeufs fraîchement pondus après une exposition aux boules de naphtaline de 7 jours, les boules étant conservées dans des conteneurs fermés contenant des pois chiche, des haricots mungo, des lentilles ou du niébé. Une étude ultérieure a montré qu'il n'y avait aucune émergence d'adultes, même après une exposition de 48 heures aux boules de naphtaline, sur les graines de pois chiche portant des oeufs fraîchement pondus. La durée efficace d'exposition à la naphtaline varie en fonction des larves cachées et de la mortalité des nymphes, autant que de la légumineuse. Après une exposition maximale de 144 heures, des larves et des nymphes, par rapport aux témoins, l'émergence des ravageurs adultes sortant des grains exposés de niébé, de pois chiche, de haricots mungo ou de lentilles était presque nulle. 100 % d'adultes sont morts après une exposition de 24 heures aux boules de naphtaline dans des conteneurs renfermant des pois chiche. Les grains récupérés immédiatement après exposition de 60 jours à la naphtaline n'ont pas semblé présenter de toxicité étant donné l'absence de grande différence ($P > 0,05$) dans le nombre d'oeufs pondus et les adultes ayant émergé de ces grains récupérés autant que dans les témoins. Aucune différence n'a été constatée ($P > 0,05$) entre le pourcentage de germination des graines de légumineuses soumises au traitement et les témoins après 60 jours d'exposition. L'exposition pendant 10 à 15 jours d'un kg de pois chiche infesté avec quatre boules de naphtaline a éliminé complètement les bruches ainsi que la croissance fongique pendant une durée minimale de sept mois dans tous les types de conteneurs étanches.