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Efficacy of Plant Dusts, Oils and Indigenous Materials against Stored Pulse Beetle *Callosobruchus chinensis* L.

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Abstract

The experiments were conducted to study the efficacy of plant dusts, oils and indigenous materials against pulse beetle (*Callosobruchus chinensis* L.) on mungbean (*Vigna radiata* L. Wilczek) seeds at the laboratory of Grain Legumes Research program, Rampur, Chitwan. The botanicals Timur (*Zanthoxylum armatum* DC), Camphor balls (*Cinnamomum camphora* L.), Tobacco (*Nicotiana tabacum* L.), Black peeper (*Piper longum* L.) were dried under shade, to avoid loss of active ingredients, until the moisture content was completely removed from those materials. Well-dried botanical materials were made in powder form using grinder machine. Similarly, plant derived oils Neem oil (*Azadirachta indica* A. Juss), Soybean oil (*Glycine max* L. Merr), Mustard oil (*Brassica juncea* L.), Coconut oil (*Cocos nucifera* L.) and Sesame oil (*Sesamum indicum* L.) were purchased from market. Ten pairs of adults were transferred to each plastic bottle with perforated lead of capacity 1 kg containing 300 g botanicals (dust and oils) treated seeds using brush. The total numbers of treatments were 11 including one control following completely randomized design with four replications. The concentration of plant dust and plant oil was maintained 2g/kg and 5ml/kg of seed respectively during the treatment of seed. The efficacy was evaluated by considering total number of adult emergence, final weight, percent weight loss, germination percentage and moisture percentage of mungbean seeds up to 9 month of storage. Among botanicals tested, camphor and tobacco dust @ 2 g/kg of seed from powder form and plant oils-neem, sesamum and soybean, respectively @ 5 ml/kg of seed can be explored as excellent alternative over the poisonous pesticide for the management of *C. chinensis* L. in storage of mungbean and other pulses. The extracts of camphor and neem seed had no adverse effects on seed germination up to nine months of storage.

Keywords: Efficacy; Plant dusts; oils; indigenous materials; pulse beetle

Introduction

Pulses have a prominent place in daily diet and they are rich source of vegetable protein, minerals and vitamins. They are of special significance of the people in developing countries like Nepal, who can hardly afford animal protein in adequate quantities (GLRP, 2013). The pulse seeds suffer a greater damage during storage due to insect attack (Sharma, 1989). Among the insect pests attacking stored products, pulse beetle *Callosobruchus chinensis* L. is reported to be the major pest infesting all types of pulses both in the field and in storage mostly. *Callosobruchus chinensis* L. is cosmopolitan and a serious pest of pulses and has also been reported attacking cotton seed, sorghum and maize (Ahmed *et al*., 2003). Due to invasion of the beetle, deterioration in quality and quantity of stored pulse is high in terai and foot hill region of Nepal (Paneru and Shivakoti, 2001). Since use of insecticides is not advised on food grains directly, it has been practice in the past to use plant extract as grain protectants (Jilani *et al.*, 1988). Neem powder and its extract works as repellent and has been reported by several researchers against *C. chinensis* L. and others (Said, 2004; Reddy and Singh, 1998; Bhuiyah *et al.*, 2002). Tripathy *et al.* (2001) tested effect of plant powders and extracts against *C. chinensis* L. attacking black gram. Al-Lawati *et al.* (2002) tested the potential of eight plant extracts against oviposition, adult emergence and mortality of *C. chinensis*. Gautam *et al.* (2000) evaluated the effect of nine edible plant products *i.e.*,aonla, black pepper, bitter gourd, clove, cinnamon, fenugreek, ginger, red chilies and tumeric to control chickpea beetle attack to stored chickpea. Aslam *et al.* (2004) tested the bio-efficacy of ten plant materials including leaves of olive, tea, bhang, elephanta, neem, dharek and fruits of garlic, cloves, black pepper and red chilies in ground form against biology and life span of *C. Chinensis* L. At present, pest control measures in storage rely on the use of synthetic insecticides and fumigants. Their indiscriminate use in the storage, however, has led to a number of problems including insect's resistance, toxic residues in food grains (Fishwick, 1988), environmental pollution (WMO, 1995) and increasing costs of application. Manmade chemicals have many harmful effects (Purohit and Vyas, 2005). In view of these problems together with the upcoming WTO regulations, there is a need to restrict their use globally and implement safe alternatives of conventional insecticides and fumigants to protect stored grains from insect infestations (Yusof and Ho, 1992; Subramanyam and Hagstrum, 1995). There is no doubt that botanical insecticides are an interesting alternative to insect pest control, and on the other hand only a few of the more than 250,000 plant species on our planet have been properly evaluated for this purpose (Khalequazzaman and OsmanGoni, 2009) . Plant material based (botanicals) insecticides are target specific, non-toxic to human and beneficial organisms, less prone to insect resistance and resurgence, biodegradable and less expensive and are promising grain protectants. Plant materials constitute a rich source of bioactive chemicals (Wink, 1993); hence they could lead to the development of new classes of safer insect control agents. Use of plant for infestation control in stored grains therefore seems to offer desirable solutions, especially in developing tropical countries where plants are found in abundance everywhere throughout the year.

Pulse beetle being an internal feeder can't be controlled easily with chemical insecticides. It is also not advisable to mix insecticides with food grains. Fumigation being the most effective method can't be practiced in our villages because the storage structures are not airtight and are mostly built inside the residential areas. Plant and other local materials which are being traditionally used by some farmers are less costly, easily available, safer, don't do any hazards using in the stores and appears to be the most promising as grain protectants. Keeping these views, the present experiment was designed to investigate the efficacy of botanicals and indigenous materials against stored pulse beetle *Callosobrochus chinensis* L.

Materials and Methods

One kilogram, clean and non-damaged mungbean variety "Pratikshya" was used for insect rearing, which was obtained from the GLRP, Rampur, Chitwan, Nepal. The bulk of mungbean seeds were fumigated with celphos at recommended dose (1 tablet/ton of seeds) for 72 hours. The seed lot was normalized with exposure to air thoroughly and sun dried. Initial moisture content of the seed was recorded with the help of moisture meter (Multigrain Tester). Similarly, initial germination test was also performed using blotting paper method. At the time of experimentation, the moisture content of seed was 12% and the germination was 99%.Maintenance and mass rearing of insect was performed at room temperature (32±2^{o}C) in Entomology Laboratory at GLRP. The culture was produced as pure culture for the inoculation into each experiment. The different botanicals used in the research were Nepal pepper-Timur (*Xanthoxylum armatum* DC*)*, Camphor balls (*Cinnamomum camphora* Linn.*)*, Tobacco (*Nicotiana tabacum* Linn.), Black pepper (*Piper longum* Linn.) as well as Neem oil (*Azadirachta indica* A. Juss), Soybean oil (*Glycine max* L. Merr*)*, Mustard oil (*Brassica juncea* Linn.), Coconut oil (*Cocos nucifera* Linn.*)* and Sesame oil (*Sesamum indicum* Linn.*)* were purchased from nearby market. All the botanicals were dried under shade, to avoid loss of active ingredients, until the moisture content was completely removed. Well-dried botanical materials were made in powder form using grinder machine.

Adult preparation and transfer of C. chinensis

Ten pairs of adults were transferred using brush to each plastic bottle of capacity 1 kg containing 300 g botanicals treated seeds. In the same way plant oil was mixed with seeds. After through mixing each treated seed lot of 300 g were kept in plastic bottle having 1 kg capacity with perforated lead. The total numbers of treatments were 11 including one control following completely randomized design with four replications. The concentration of plant dust and plant oil was maintained 2 g/kg and 5 ml/kg of seed, respectively during the treatment of seed. The number of dead adults were removed and counted each day from next day until complete death and removal of adults in each treated container. Germination percent of the seeds was taken prior to the experimental setup and also at end of the experiment (9 month after storage) using blotting paper method. For the assessment of percent weight loss, 100 grain samples were taken randomly at final recording followed by counting damaged and undamaged grains. Weights of damaged and undamaged grains were recorded with the help of electronic balance. Initial weight, final weight, total adult emerged, percent weight loss, percent germination, percent moisture were recorded.

All data were analyzed statistically using MSTAT-C computer software package. Significant treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% levels. In all cases, a significance level of p< 0.05 was used unless otherwise stated.

Results and Discussion

Plant extracts (dust)

Botanicals use in powdered form showed significant differences (p<0.05) in the mortality of pulse beetle (Table 1). The trend of mortality significantly increased after 24 hour of treatment. The highest adult mortality was observed on the seeds treated with camphor dust (93.35%) followed by tobacco (89.15%) and the lowest on seeds treated with match sticks powder (7.5%). Nepal pepper (*X. armatum*) dust showed intermediate effect in causing mortality of adults (57.1%). However, all the treatments were superior to control (Fig. 1).

Plant dust showed highly significant effects on total number of adult emergence, final weight, percent weight loss, germination percentage and moisture percentage of mungbean seeds up to 9 month of storage (Table 2). The total number of adults emergence of *C. chinensis* were significantly lower in mungbean seeds treated with camphor dust (19.20 adults/seed) followed by seeds treated with tobacco dust (33.50 adults/seed) and black pepper (42.50 adults/seed) as compared to control (2963.00 adults/seed). But, there was no significant difference observed in match stick dust treated seeds and control in case of adult emergence.

Final seed weight was significantly higher when treated with camphor dust (293.00 g) followed by seeds treated with tobacco dust (282.30 g). Similarly, weight loss was also significantly lower in seed treated with camphor and tobacco dusts, i.e. 2.32% and 5.90%, respectively. Germination percent was found higher in camphor dust treated seeds (94.67 %) with low moisture percent content (13.73%), while seeds had higher moisture in control (18.46%), i.e. without any treatment (Table 2).

Table 1: Effect of plant extracts (dust) to adult mortality of *C. chinensis* at GLRP, Rampur, Chitwan, 2013

Treatments	Mortality of pulse beetle, *C. chinensis* (No)					
(Dust-2 g/kg seed)	1DBT	24HAT	48HAT	72HAT	Mean	Mortality (%)
Black pepper	20.00	†12.25^{c}	13.75^{b}	14.75^{b}	13.58	67.90
(*P. longum*)		(3.57)	(3.77)	(3.91)		
Match sticks	20.00	1.00^{e}	1.25^{d}	2.25^{c}	1.50	7.50
		(1.22)	(1.31)	(1.65)		
Nepal pepper	20.00	7.00^{d}	12.00^{c}	15.25^{b}	11.42	57.10
(*X. armatum*)		(2.73)	(3.53)	(3.96)		
Tobacco	20.00	16.00^{b}	17.75^{a}	19.75^{a}	17.83	89.15
(*N. tabacum*)		(4.06)	(4.27)	(4.50)		
Camphor	20.00	17.50^{a}	18.75^{a}	19.75^{a}	18.67	93.35
(*C. camphora*)		(4.24)	(4.39)	(4.50)		
Control	20.00	0.00^{f}	0.00^{e}	0.25^{d}	0.08	0.40
		(0.71)	(0.71)	(0.84)		
F-Test		**	**	**		
LSD (≤0.05)		0.17	0.15	0.23		
CV%		4.13	3.53	4.80		

† Means of four replications. Mean values in column with the same superscript are not significantly different by DMRT $_{(P<0.05)}$. Figures in the parenthesis indicate the square root transformed value. DBT- Days before treatment, HAT- Hour after treatment, LSD- Least significant difference, CV- coefficient of variation, **- Highly significant (p>0.001)

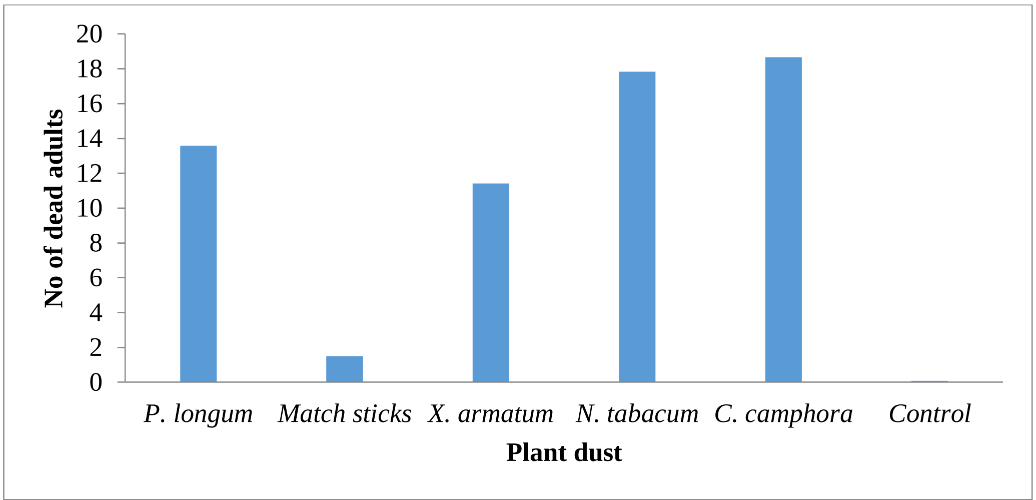

Fig. 1: Effect of plants dust to mean no of dead adults of *C. Chinensis* at GLRP, Rampur, Chitwan, 2013

Table 2: Effect of plant dust on total adult emerged of *C. chinensis*, final seed weight, weight loss, germination and moisture on mungbean seed at GLRP, Rampur, Chitwan, 2013

Treatments (Dust -2 g/kg seed)	Initial seed wt (g)	Final seed wt. (g)	Total adult emerged (No)	Seed wt. loss (%)	Seed Germ(%)	Seed moisture (%)
Black pepper (*P. longum*)	300.00	†281.90^{b}	42.50^{b}	16.02^{c}	83.43^{c}	15.56^{c}
Match stick	300.00	253.30^{c}	2642.00^{a}	15.58^{b}	73.88^{e}	17.13^{b}
Nepal Pepper (*X. armatum*)	300.00	263.20^{c}	710.50^{b}	12.27^{b}	80.34^{d}	16.75^{e}
Tobacco (*N. tabacum*)	300.00	282.30^{b}	33.50^{c}	5.90^{c}	86.31^{b}	14.93^{c}
Camphor (*C. camphora*)	300.00	293.00^{a}	19.25^{b}	2.32^{c}	94.67^{a}	13.73^{d}
Control	300.00	237.30^{d}	2963.00^{a}	20.92^{a}	71.26^{f}	18.46^{a}
F-Test		**	**	**	**	**
LSD (≤0.05)		10.57	801.40	3.52	2.57	0.84
CV%		2.65	50.49	22.58	2.12	3.52

† Means of four replications. Mean values in column with the same superscript are not significantly different by DMRT $_{(P<0.05)}$. Wt- Weight, Germ- Germination, g-gram, LSD- Least significant difference, CV- coefficient of variation, **- Highly significant (p>0.001)

The result of this study is in accordance with the findings of may authors Singh *et al.* (1996), Kumari and Singh (1998), Ignatowicz *et al*., (1995), Javaid and Poswal (1995), Aslam *et al.* (2002), Umrao and Verma (2002), Singh (2003) and Boeke *et al.* (2004) who concluded that plant dusts were proved to be equally effective against bruchids in respect of control of number of eggs laid, number of adults emergence, reduction in damage to grain by the pest, weight loss, moisture content and even in germination of the seed. Budavari (1996) reported that camphor is a natural compound derived from the camphor tree (*C. camphora*), which has a familiar and penetrating odor and a slightly bitter and cooling taste. Obeng *et al.* (1998) reported that camphor is very good repellent to stored products beetles.

Relationship between number of adult emergence and seed weight loss

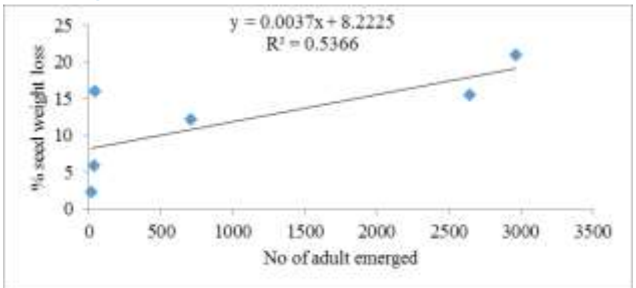

Fig. 2: Relationship between total number of adult emerged and percent seed weight loss of mungbean at GLRP, Rampur, Chitwan, 2013

A positive linear correlation between total number of adult emergence of *C. Chinensis* and percent seed weight loss of mungbean seed was observed after 9 month of seeds storage treated with plant dusts. Equation Y= 0.003X + 8.222 and R^2= 0.536 gave the best fit, indicating higher the number of beetle emergence and higher the seed weight loss (Fig. 2).

Effect of seed treatment with plant oils

Various plant oil treated seeds showed highly significant difference (p< 0.05) on the mortality of pulse beetle (Table 3). The trend of beetle mortality significantly increased after treatment interval of 24 hour except in soybean seeds oil after 72 hour of treatment. The highest dead adult mortality was observed in the seed treated with neem oil (99.6%) followed by soybean oil *(*87.9%) and the lowest dead adults were observed in control (0.40%). Sesame, mustard and coconut oil was statistically same with respect to adult mortality of *C. chinensis* (Fig. 3).Plant oils showed highly significant effect on the number of adult emergence, final seed weight, percent weight loss, germination and moisture percentage of mungbean seeds after 9 months of storage (Table 4). The total number of *C. Chinensis* adults emergence were significantly lower from mungbean seeds treated with neem oil (15.25 adults/seed) followed by seeds treated with sesame oil (23.50 adults/seed) as compared to the control (2896.00 adults/seed). Final weight was also significantly higher for seeds treated with neem oil (295.50 g) and the lowest weight loss (1.49%) followed by sesame oil treated seeds weight (292.10 g) and weight loss (2.64%), respectively. Germination was higher in neem oil treated seed (97.83%) with low moisture content (11.00%). Significantly higher moisture was recorded in control seed (19.23%), i.e. without any treatment (Table 4).

Table 3: Effect of plant oils on adult mortality of *C. chinensis* at GLRP, Rampur, Chitwan, 2013

Treatments (Oil-5 ml/kg seed)	1DBT	Mortality of pulse beetle, *C. chinensis* (No) 24HAT	48HAT	72HAT	Mean	Mortality (%)
Soybean (*G. max*)	20.00	†18.25[b] (4.33)	19.50[a] (4.47)	15.00[a] (3.57)	17.58	87.90
Sesame (*S. indicum*)	20.00	16.00[c] (4.06)	17.75[b] (4.27)	20.00[a] (4.53)	17.92	89.60
Mustard (*B. juncea*)	20.00	15.75[c] (4.03)	18.00[b] (4.30)	20.00[a] (4.53)	17.92	89.60
Coconut (*C. nucifera*)	20.00	15.50[c] (4.00)	18.25[b] (4.33)	20.00[a] (4.53)	17.92	89.60
Neem (*A. indica*)	20.00	19.75[a] (4.50)	20.00[a] (4.53)	20.00[a] (4.53)	19.92	99.60
Control	20.00	0.00[d] (0.71)	0.00[c] (0.71)	0.25[b] (0.84)	0.08	0.40
F-Test		**	**	**		
LSD (≤0.05)		0.14	0.08	1.17		
CV%		2.62	1.56	20.95		

† Means of four replications. Mean values in column with the same superscript are not significantly different by DMRT $_{(P<0.05)}$. Figures in the parenthesis indicate the square root transformed value. DBT- Days before treatment, HAT- Hour after treatment, LSD- Least significant difference, CV- coefficient of variation, **- Highly significant (p>0.001)

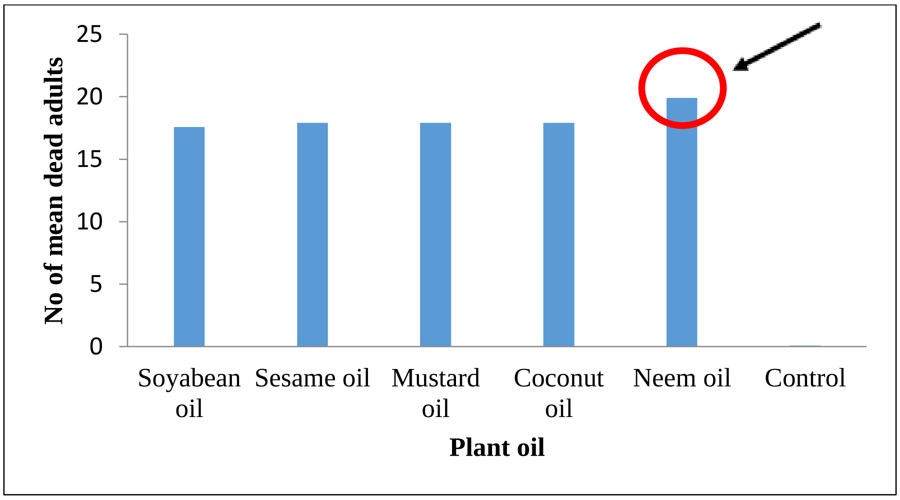

Fig. 3: Effect of plants oil to mean no of dead adults of *C. chinensis* at GLRP, Rampur, Chitwan, 2013

Table 4: Effect of plant oils on adult emergence of *C. chinensis*, final seed weight, seed weight loss, germination and moisture on mungbean seeds at GLRP, Rampur, Chitwan, 2013

Treatments (Oil- 5 ml/kg seed)	Initial seed wt. (g)	Final seed wt. (g)	Total adult emerged (No)	Wt. loss (%)	Seed Germ[n] (%)	Seed moisture (%)
Soybean (*G. max*)	300.00	†290.50[bc]	28.25[b]	3.16[cd]	94.94[bc]	13.21[c]
Sesame (*S. indicum*)	300.00	292.10[b]	23.50[b]	2.64[d]	96.53[ab]	11.95[d]
Mustard (*B. juncea*)	300.00	285.60[d]	55.25[b]	4.79[b]	94.65[bc]	14.05[b]
Coconut (*C. nucifera*)	300.00	288.30[a]	45.50[b]	3.88[bc]	93.94[c]	11.62[de]
Neem (*A. indica*)	300.00	295.50[a]	15.25[b]	1.49[e]	97.83[a]	11.00[e]
Control	300.00	234.20[e]	2896.00[a]	21.94[a]	73.58[d]	19.23[a]
F-Test		**	**	**	**	**
LSD (≤0.05)		13.37	126.90	1.12	1.96	0.83
CV%		0.81	16.73	11.97	1.44	4.12

† Means of four replications. Mean values in column with the same superscript are not significantly different by DMRT $_{(P<0.05)}$. INSWT- Initial Seed Weight, Wt- Weight, % - Percentage, Germ[n]- Germination, g-gram, LSD- Least significant difference, CV- coefficient of variation, **- Highly significant (p>0.001)

Relationship between total number of adult emergence and seed weight loss

A positive linear correlation was observed between total number of adults emerged of *C. chinensis* and percent seed weight loss of plant oil treated mungbean seeds after 9 months of storage with equation Y= 0.006X + 2.966 and R^2= 0.982 showing the best fit (Fig. 4). This clearly shows that as the number of adult emergence increases, seed weight loss also increases in proportionately.

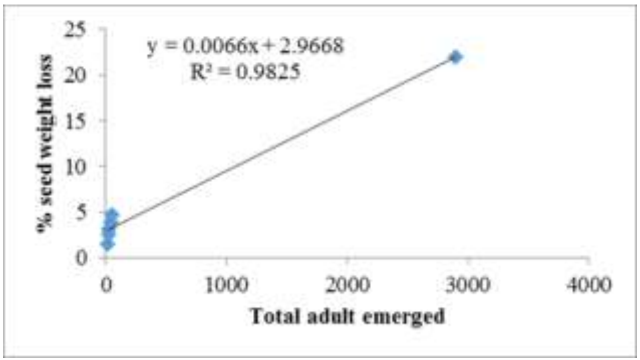

Fig. 4: Relationship between total adult emergence of *C. chinensis* and percent seed weight loss of plant oil treated mungbean at GLRP, Rampur, Chitwan, 2013.

Singh *et al.* (1996) reported that the vegetable oil is active against eggs and larvae and cause unable to oviposit or cause mortality of the adult bruchids. Only small amount of oil is needed to preserve the grains for months (1-5 ml/kg of grains). Credland (1992) examined the structure of bruchid egg and suggested that the funnel structure at the posterior pole of bruchid eggs may be the major route for gaseous exchange. It was proposed that application of oil to bruchid egg might occlude the funnel, and thus lead to the death of the developing insect by asphyxiation. Dohareye*t al.* (1990) reported that sesame oil 0.5% (w/w) applied to mungbean reduced the number of eggs laid by *C. maculatus* and prevented F1 emergence. Ali *et al.* (1983) reported that sesame oil @ 1 ml /100g applied to chickpea caused 100% mortality of adult *C. chinensis* within 3 days and completely prevented egg laying and the emergence of F1 adults. The findings of Jacob and Sheila (1990) and Uvah and Ishaya (1992) showed all plant oils tested caused 60% mortality of pulse beetle after 3 days but neem and sesame oil at 1 ml/100 g of grains were found to be the most effective. Similar studies by Khaire *et al.* (1992) also supported the present findings that adult emergence was completely prevented by neem oil. Minimum grain loss was also noted with neem oil at 1% level up to 100 days after treatment. There were no adverse effects of these oils on seed germination. Choudhary (1990) also concluded that neem, groundnut, castor, soybean and sesame oils at 0.5 and 1.0 ml/100 g of chickpea grains that reduced the damage of pulse beetle. Neem and groundnut oils were also effective at 0.25 ml/100 g grains. The result obtained was completely in accordance with the findings of Bhatnagar *et al.* (2001) who tested the efficacy of plant oils against *C. chinensis* attacking cowpea. Plant oils groundnut, sesame, soybean, mustard and neem oils (at 10 ml/kg of seed) as repellent, ovipositional deterrent and ovicidal agents against this beetle were investigated in the laboratory. All the oils evaluated were effective however neem oil appeared to be the most effective where as all the oils except neem oil exhibited reduction in efficacy with the delay in treatment time. Ahmad *et al.* (2003) showed that neem and sesame oils could control the larvae inside the bean cotyledons indicating that oils might penetrate into cotyledon and chemically inhibit or kill the larvae inside. It was concluded that earlier developmental stages were more susceptible than the later one at the depth of the larval position in the bean and its tolerance might be responsible for the age-dependant effect of oils.

Conclusion

Among botanicals tested, camphor and tobacco dust @ 2 g/kg of seed from powder form and plant oils- neem, sesamum and soybean, respectively @ 5 ml/kg of seed can be explored as excellent alternative over the poisonous pesticide for the management of *C. chinensis* in storage of mungbean and other pulses. It is hoped that they will reduce the development of pest resistance against malathion and detrimental effects of chemical fumigants used in storage structures.

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