Evaluation of Some Essential Oils against Pulse Beetle (Callosobruchus chinensis L.) in Pea Seeds

Abstract
The present investigations on “Evaluation of some essential oils against pulse beetle (Callosobruchus chinensis L.) in pea seeds” were carried out during 2014-15 in the Department of Seed Science and Technology, College of Horticulture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni–solan (H.P.). The essential oils of camphor, wild marigold, cone-bearing sage, eucalypts, lemongrass and sweet flag at 2.5 ml/kg, 1.25 ml/kg, 0.60 ml/kg and 0.30 ml/kg (v/w basis) were evaluated against C. chinensis L infesting pea seeds. Among the six essential oils, sweet flag possessed reasonably high and immediate toxicity resulting in 78.33 percent and 96.67 percent (2.5 ml/kg and 1.25 ml/kg doses respectively) mortality in 1 and 3 days after treatment. After 7 days, cent per cent mortality was observed in seeds coated with sweet flag essential oil followed by eucalypts (85.83%), cone-bearing sage (77.50%), camphor (74.17%), lemongrass (71.66%) and wild marigold (61.67%) in descending order. After 10- days of exposure, eucalypts essential oil also resulted in complete kill even at the lowest dose (0.30ml/kg). On day -15, mortality in control had substantially increased to 67.50 percent. Egg laying was minimal on sweet flag essential oil treated pea seeds (5.25 eggs/5 females) on 7th – day of observations. In untreated pea seeds, increases in egg laying was negligible in 20 – days observations (7th day 94.08-20th day 101.92). Progeny development from the eggs laid on pea seed treated with sweet flag essential oils was (1 beetle).

Keywords: Callosobruchus chinensis; Fecundity; Mortality; Pea seeds; Essential oil

Introduction
The successful production of any crop depends on the quality of seeds sown. Maintenance of high seed germination and vigour from harvest until planting is of utmost importance in a seed production programme. Pea (Pisum sativum L.) is an important vegetable crop of Himachal Pradesh. It is grown in an area of about 23900 ha with annual production of 271060 metric tonnes [1]. One of the major constraints for its production is the attack by insect- pests in the field as well as in storage. Several bruchid species attack cereals and pulses in the store and cause loss up to 10-15 per cent with a germination loss ranging from 50-92 per cent [2]. Losses due to insect infestation are the most serious problem in grain storage, particularly in the developing countries, where poor sanitation and use of inappropriate storage facilities encourage insect attack [3,4]. Stored product insects cause post-harvest losses up to 9 per cent in developed countries and more than 20 per cent in developing countries [5]. According to an estimate, the overall annual damage caused by stored grain insect pests accounts for 10-40 per cent worldwide annually [6]. In India, food grain losses during storage at the farm level approximate 10 per cent of the total production. Inspite of improved storage structures and modern chemical and physical control techniques employed for the safe storage of stored grains, 70-90 per cent of food grain is still stored for six months to a year at farmer’s level in traditional storage structures [7]. In such a critical situation, protection of stored seed grains from insect infestation is quite essential.

Among the important insect pests that infest the pea seeds in storage is the pulse beetle, Callosobruchus chinensis L. (Bruchidae: Coleoptera) which causes substantial losses to the pulses in the storage [8-10] though the initial infestation occurs in the field itself. It is a cosmopolitan and serious pest of peas, mung, cowpeas and lentil and has also been reported attacking cotton seed, sorghum and maize [11]. Its active period is between March and October/November [2] and when such seeds harvested and stored, the pest population increases rapidly and results in total destruction within a short duration of 3-4 months. Only grubs are damaging stages for the storage grains. These make holes in the grains which become unsuitable for human consumption, production of sprout and also lose its market value [12]. It causes weight loss, decreased germination potential and reduction in commercial value of seed [14]. Its damage generally starts in ripened pods in the field from where it is carried over to storage godowns. Adult females lay eggs on seeds and the emerging grubs bore in to seeds and feed on endosperm. Consequently seeds lose their viability and nutritional value. The life cycle is completed in 25-34 days during summer whereas, it takes 40-50 days in winter [15].
In order to keep stored seeds free from insect-pests infestations various synthetic pesticides are used. Although chemical insecticides are effective, but their indiscriminate use has led to residual toxicity, insecticide resistance, environmental pollution and adverse effects on food besides side effects on humans [16-18]. Methyl bromide and phosphate fumigants have been used for decades to control stored pests [6,19]. Growers are moving away from using methyl bromide as post-harvest fumigant because of its ozone-depleting nature. Ozone depleting nature of methyl bromide has led to restriction of its use, and the Montreal protocol of United Nations Environment Programme (UNEP) recommend the phasing out of methyl bromide by 2015 in developing countries [20]. Phosphine resistance is becoming more common [21-23] and is a matter of considerable concern. Therefore, there is a need of some other alternatives to chemical pesticides and fumigants to protect stored grains from insect-pests infestations.

Material and Methods

Raising of test insect culture

The pure culture of pulse beetle (Callosobruchus chinensis L.) was maintained under laboratory conditions throughout the year. For this purpose pea seeds cv. PB-89 were heat sterilized at 55°C for 4 hours in an oven. These sterilized seeds were kept in sterilized jars of half kg capacity (Plate-1). In these jars ten pairs of freshly emerged adults of C. chinensis were released (Plate-2). The jars were covered with muslin cloth and kept in BOD incubator maintained at 27±1°C temperature and 70±5% relative humidity for further multiplication of beetles.

Selection of essential oils: Six plant essential oils viz. Camphor (Cinnamomum camphora L.), Wild marigold (Tagetes minuta L.), Cone-bearing sage (Meriandra strobilifera B.), Eucalyptus (Eucalyptus sp.), Lemongrass (Cymbopogon citratus L.). Sweet flag (Acorus calamus L.) were used for their insecticidal activity against C. chinensis and then on their effect on seed quality parameters (Plate-3). The plant material was collected locally, shade dried and essential oils were extracted with the help of clevenger apparatus by hydro-distillation (Plate-4) in the Department of Forest Products.

Evaluation of plant essential oils at different doses: Each essential oil was taken at different doses viz. 2.5 ml/Kg 1.25ml/Kg, 0.60 ml/Kg and 0.30 ml/kg in separate plastic container of 250cc capacity containing 100 g of sterilized seeds of pea with three replications. Contents were thoroughly mixed in plastic containers by vigorous shaking. In control no essential oil was mixed. Five pair of freshly emerged adults of pulse beetle were then released in each container. These containers were closed by muslin cloth tightly secured by rubber band. The experiment was carried out at room temperature (Plate-5). During the experimental period the average minimum and maximum temperature (°C) was 14.5°C and 26.8°C respectively. There were seven treatments including control with three replications. The effect of treatment was studied on different biological parameters as per details given below:

Efficacy of different essential oils on adult mortality of C. chinensis: In order to determine the effect of plant essential oils on the life span of adults, the mortality of adult beetles released on treated seeds was recorded at different doses at 1, 3, 7, 10, and 15 days, till mortality of all the adults released in each treatment.

Effect of essential oils on fecundity and progeny development in C. chinensis: The effects of different essential oils of all six plant species were recorded on fecundity of C.chinensis on day-2 and day-20 of release of adults and further progeny development after two months of release of adults. Egg laying data of progeny developed on treated seeds was recorded, and compared with untreated individuals.

Statistical Analysis

The data emanating from the above experiments was subjected to statistical analysis through two factor and three factor Completely Randomized Design after applying proper transformation. The significance of each treatment was calculated as suggested by Cochran and Cox.

Results

Data contained in Table 1 reveal that essential oils resulted in variable mortality of adults of C. chinensis with maximum mortality in sweet flag essential oil and minimum in untreated control.

The maximum mortality (78.33%) observed in seed coated with sweet flag essential oil was significantly superior over rest of the essential oils. Next best treatment was camphor (16.67%) which was statistically at par with par with lemongrass (16.67%), eucalypts (14.17%) and cone-bearing sage (15%). Mortality in pea seeds treated with wild marigold (8.33%) showed least mortality. All the treatments were superior over control. Mortality in pea seeds treated with sweet flag essential oil at 2.5 ml/kg resulted 100 per cent mortality which was statistically at par with its 1.25 ml/kg (100.00%). Pea seeds treated with camphor essential oil at 1.25 ml/kg, cone-bearing sage (0.60 ml/kg), wild marigold (2.5 ml/kg), eucalypts (0.60 ml/kg) recorded equal mortality (13.33 % kill). Mortality of pulse beetle significantly decreases with decrease in doses (2.5ml/kg;34.29; 0.30ml/kg; 8.57 % kill).

Among various essential oils sweet flag gave best results (96.67% overall kill) which was statistically superior over camphor (43.33%) and lemongrass (35%). Mortality in pea seeds treated with cone-bearing sage (25.83%), eucalypts (21.67%) and wild marigold (18.33%) was statistically at par with each other and were significantly superior over control (4.16%). Mortality in pea seeds treated with 1.25 ml/kg dose of lemongrass (33.33%) was at par with its 0.60 ml/kg and 0.30 ml/kg doses (0.60 ml/kg :26.67%; 0.30 ml/kg :16.67%) and 1.25 ml/kg, 0.60 ml/kg doses of eucalypts (1.25 ml/kg :26.67%; 0.60 ml/kg :20%); 1.25 ml/kg and 0.60 ml/kg doses of cone-bearing sage (1.25 ml/kg :26.66% ; 0.60 ml/kg ;23.33%), 2.5 ml/kg, 1.25 ml/kg and 0.60 ml/kg of wild marigold (2.5 ml/kg :23.33%; 1.25 ml/kg :20.00% ; 0.60 ml/kg :16.67%). Mortality recorded in pea seeds treated with essential oils was significantly superior over control (4.16%).

Highest mortality was observed in pea seeds coated with sweet flag essential oil (100%). The next best treatment was eucalypts (85.83%) followed by cone-bearing sage (77.50%) and camphor (74.17%) which was statistically at par with each other.

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Lemongrass essential oil resulted 71.67 per cent mortality which was significantly superior over wild marigold (61.67%) and both were significantly superior over control (11.66%). Mortality in pea seeds treated with eucalypts at 2.5 ml/kg dose (96.67%) was statistically at par with its 1.25 ml/kg dose (86.67%), lemongrass at 2.5 ml/kg (93.33%), cone-bearing sage at 1.25 ml/kg dose (93.33%) and 2.5 ml/kg, 1.25 ml/kg and 0.60 ml/kg dose of camphor (2.5 ml/kg : 93.33 ; 1.25 ml/kg : 86.66% ; 0.60 ml/kg : 83.33%) (Table 2).

**Table 1A:** Effect of pea seed treatment with different doses of essential oils of some plant species on mortality of *C. chinensis* beetles at different days after treatment.

| Plant species | Day-1 | Mean | Day-3 | Mean | Day-7 | Mean |
|---------------|-------|------|-------|------|-------|------|
| Camphor       | 33    | 37   | 31    | 30   | 30    | 31   |
| Wild marigold | 10    | 10   | 10    | 10   | 10    | 10   |
| Cone-bearing sage | 20  | 20   | 20    | 20   | 20    | 20   |
| Lemongrass    | 40    | 40   | 40    | 40   | 40    | 40   |
| Sweet flag    | 100   | 100  | 100   | 100  | 100   | 100  |
| Control       | 0     | 0    | 0     | 0    | 0     | 0    |
| Mean          | -35   | -25  | -35   | -25  | -35   | -25  |

**Table 1B:** Effect of pea seed treatment with different doses of essential oils of some plant species on mortality of *C. chinensis* beetles at different days after treatment.

| Treatment      | Day-10 | Day-15 | Dose (ml/kg) |
|----------------|--------|--------|--------------|
| Camphor        | 100.00 | 100.00 | 2.5          |
| Wild marigold  | 100.00 | 100.00 | 2.5          |
| Cone-bearing sage | 100.00 | 100.00 | 2.5          |

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### Table 2: Effect of essential oils on oviposition by *C. chinensis* on treated pea seeds.

| Treatments          | Day-7                   | Day-20                   |
|---------------------|-------------------------|--------------------------|
|                     | 2.5 (2.74)              | 0.60 (2.64)              | 0.60 (2.64)              | 0.30 (2.64)              | 0.30 (2.64)              | Mean (2.64)              |
| Camphor             | 6.67 (2.74)             | 32.67 (2.64)             | 47.00 (2.64)             | 75.00 (2.64)             | 40.33 (2.64)             | 9.00 (2.64)              | 36.33 (2.64)             | 49.33 (2.64)             | 78.33 (2.64)             | 43.25 (2.64)             |
| Wild Marigold       | 17.33 (4.19)            | 29.00 (4.19)             | 37.00 (4.19)             | 49.33 (4.19)             | 33.16 (4.19)             | 23.33 (4.19)             | 35.33 (4.19)             | 40.67 (4.19)             | 52.67 (4.19)             | 38.00 (4.19)             |
| Cone-bearing sage   | 22.00 (4.75)            | 31.33 (4.75)             | 36.67 (4.75)             | 47.95 (4.75)             | 34.41 (4.75)             | 24.33 (4.75)             | 36.00 (4.75)             | 42.67 (4.75)             | 49.33 (4.75)             | 38.08 (4.75)             |
| Eucalypts           | 47.00 (6.89)            | 59.00 (6.89)             | 72.33 (6.89)             | 92.67 (6.89)             | 67.75 (6.89)             | 49.67 (6.89)             | 62.33 (6.89)             | 73.00 (6.89)             | 93.33 (6.89)             | 69.58 (6.89)             |
| Lemongrass          | 3.33 (2.08)             | 6.67 (2.08)              | 3.83 (2.08)              | 12.67 (2.08)             | 7.75 (2.08)              | 3.33 (2.08)              | 9.00 (2.08)              | 11.67 (2.08)             | 14.33 (2.08)             | 10.08 (2.08)             |
| Sweet flag          | 2.00 (1.71)             | 1.67 (1.71)              | 6.00 (1.71)              | 8.33 (1.71)              | 5.25 (1.71)              | 4.67 (1.71)              | 7.00 (1.71)              | 9.33 (1.71)              | 11.67 (1.71)             | 8.17 (1.71)              |
| Control             | 93.00 (9.66)            | 88.33 (9.66)             | 96.67 (9.66)             | 98.33 (9.66)             | 94.08 (9.66)             | 96.67 (9.66)             | 100.00 (9.66)            | 102.00 (9.66)            | 106.67 (9.66)            | 101.92 (9.66)            |
| Mean                | 27.33 (4.57)            | 35.95 (4.57)             | 43.43 (4.57)             | 54.86 (4.57)             | 40.39 (4.57)             | 30.71 (4.57)             | 41.91 (4.57)             | 46.95 (4.57)             | 58.05 (4.57)             | 44.16 (4.57)             |

Figure in parentheses are square root transformed values

CD (p=0.05)
Day: 2.72
Dose X Day: 5.43
Treatment X Day: 7.19
Treatment X Dose X Day: 14.38

Evaluation of Some Essential Oils against Pulse Beetle (*Callosobruchus chinensis* L.) in Pea Seeds.
Mortality recorded in pea seeds treated with sweet flag and eucalyptus oil was equal (both 100% kill) and both were statistically significant at par with camphor (96.67%). Next best treatment was lemongrass (88.33%) which was statistically at par with cone-bearing sage (87.50%). Lowest mortality was observed in pea seeds treated with wild marigold (78.33%) which was significantly superior over control (44.17%). Cent per cent pulse beetle mortality was observed at all dose of sweet flag and eucalyptus essential oils and 2.5 ml/kg dose of camphor (both 100%), 2.5 ml/kg; 1.25 ml/kg doses of cone-bearing sage (both 100%) and 2.5 ml/kg dose of lemongrass (100%). Mortality response (96.67%) of 0.6 ml/kg dose of camphor was statistically at par with its 0.3 ml/kg dose (90%), 2.5 ml/kg; 1.25 ml/kg dose of wild marigold (2.5 ml/kg 93.33%; 1.25 ml/kg :90.0%), cone-bearing sage at 0.60 ml/kg (93.33%) and lemongrass at 2.5 ml/kg and 1.25 ml/kg doses of lemongrass (1.25 ml/kg ; 0.60 ml/kg ; 83.33%). Pea seeds treated with wild marigold caused lowest mortality (78.33%), which was significantly superior over control (44.17%).

All the treatments were superior over control and mortality in treatments such as camphor (100%), eucalyptus (100%), lemongrass (96.67%) and wild marigold (95%) were statistically at par with sweet flag essential oil as well as with each other. Next best treatment was wild marigold (95%) and cone-bearing sage (93.33%) which was statistically at par with each other. Lemongrass at 0.30 ml/kg dose (90%) was statistically at par with 0.30 ml/kg of wild marigold (83.33%) and cone-bearing sage (80.33%). Mortality recorded at 0.60 ml/kg dose of lemongrass as well as wild marigold at same dose were equally effective (96.67% mortality). Overall result shows that mortality of pulse beetle decreases with decrease in dose (2.5 ml/kg : 95.24%; 0.30 ml/kg : 88.10%).

Effect of essential oils on fecundity of C. chinensis at different intervals and doses

Data contained in Table 3 revealed that on day 7 minimum number of eggs (5.25 eggs / 5 females) was laid by 5 pairs of C. chinensis in sweet flag essential oil coated seeds followed by lemongrass (7.75 eggs / 5 females), wild marigold (33.16 eggs / 5 female), cone-bearing sage (34.41 eggs / 5 females), camphor (40.33 eggs / 5 females) and eucalyptus (67.75 eggs / 5 females), all the essential oils were statistically different from each other.

Eucalyptus proved to be least effective (67.75 eggs / 5 females) but was superior to untreated control (94.08 eggs / 5 females). The number of eggs laid in control was very high (94.08 eggs / 5 females). Even the best proved essential oil of sweet flag differed non significantly with respect to the doses used and hence at par with each other. Overall the egg laying by 5 pairs of beetles were dose dependent as the dose increases the egg laying decreases (54.86 eggs / 5 females at 0.30 ml/kg and 27.33 eggs / 5 females at 2.5 ml/kg).

The minimum number of eggs (8.17 eggs / females) were laid by 5 pairs of beetles on seeds coated with sweet flag essential oil. Next best treatment was lemongrass (10.08 eggs / females) which was statistically superior over rest of essential oils. Among other treatments, egg laying recorded with wild marigold treated pea seeds (38.00 eggs / 5 females) was significantly at par with cone-bearing sage (30.68 eggs / 5 females) and differed significantly with rest of the essential oils. There was significant reduction in egg laying from 0.30 ml/kg to 2.5 ml/kg dose in all essential oils. Comparison of oviposition taking place on treated seeds during first seven days with the eggs laid in next 13 days reveals that there was no significant increase in oviposition in treated and control lots and whatsoever oviposition occurred, that remained restricted to first week of adult life.

Table 3: Effect of essential oils on number of beetles / 5 pairs developed after 60-days of treatment of pea seeds at different dose of essential oils.

| Treatment       | Dose (ml/kg) | 0.30 | 0.60 | 1.25 | 2.5 | Mean
|-----------------|--------------|------|------|------|-----|------
| Camphor         | 5.00 (2.31)  | 1.09 | 5.69 | 17.33 (4.49) | 36.67 | 50.74 | 0.30 | 71.67 | 25.33 | 39.67 | 56.33 | 71.67 | (5.71) | 47.42 |
| Wild Marigold   | 11.33 (3.43) | 2.31 | 30.33 (5.54) | 29.67 (5.49) | 34.67 (5.91) | 25.58 (5.09) | 74.00 |
| Cone-bearing sage | 12.33 (3.62) | 2.31 | 29.67 (5.49) | 34.67 (5.91) | 25.58 (5.09) | 74.00 |
| Eucalypts       | 29.67 (5.47) | 2.31 | 56.33 (7.53) | 71.67 (8.49) | 67.17 (8.49) | 47.42 (6.80) | 74.00 |
| Lemongrass      | 1.33 (1.34)  | 2.31 | 1.67 (1.67) | 2.33 (1.67) | 3.33 (2.96) | 3.42 (1.86) |
| Sweet flag      | 2.31 (1.46)  | 2.31 | 1.09 (1.04) | 1.67 (1.76) | 2.67 (1.76) | 0.94 (1.06) |
| Control         | 70.33 (8.43) | 2.31 | 71.67 (8.49) | 74.65 (8.65) | 72.66 (8.55) | 72.66 |
| Mean            | 18.57 (3.61) | 2.31 | 32.62 (5.19) | 48.19 (5.89) | 30.66 (4.78) | 30.66 |

Figures in parenthesis are ±SE of 5 transformed values
References

1. Anonymous (1985) International rules for seed testing. Seed Science and Technology 13: 299-335.
2. Adugna H (2006) On farm storages studies in Eritrea. African Journal of Biotechnology 5(17): 1537-1544.
3. Talukder FA, Islam MS, Hossain MS Rahman MA and Alam MN (2004) Toxicity effects of botanicals and synthetic insecticides on Tribolium castaneum (Herbst) and Rhyzopertha dominica (F.). Bangladesh Journal of Environmental Science 10(2): 365-371.
4. Talukdar FA (2005) Insects and insecticides resistance problems in post harvest agriculture. In: Proceedings of International Conference on Post harvest Technology and Quality Management in Arid Tropics, Sultan Qaboos University, Oman, pp. 207-211.
5. Philips TW (2010) Biologically based approaches in managing storage insects. In: 3rd Conference on biopesticide in food and environment security, CCS.
6. Mishra AK, Kumar R, Dubey NK, and Tripathi YB (2007) Evaluation of Chenopodium amamioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int J Food Microbiol 115(2): 159-164.
7. Chaubey MK (2013) Biological activity of Zinziber officinale (Zingiberaceae) and Piper cubeba (Piperaceae) essential oils against pulse beetle, Callosobruchus chinensis. Pak J Biol Sci 16(11): 517-523.
8. Alam MZ (1971) Pest of stored grains and other stored products and their control. The agriculture information service, Publication, Dhaka-61.
9. Borikar PS, Puri SN (1985) Damages and losses caused by Callosobruchus chinensis L to different legumes stored in different container. Agriculture Science Digest 5: 108-110.
10. Righi-Assia AF, Khell MA, Bensaad M, Righi K (2010) Efficacy of oils and powders of some medicinal plants in biological control of pea weevil (Callosobruchus chinensis, L.). African Journal of Agricultural Research 5(12): 1474-1481.
11. Ahmed KS, Itino T, Ichikawa T (2003) Duration of development stages of Callosobruchus chinensis L. (Coleoptera: Bruchidae) on adzuki bean and the effect of neem and sesame oils at different stages of their development. Pakistan Journal of Biological Science 6(10): 932-935.
12. Huignard J, Leroi B Alzouma I Germain JP (1985) Oviposition and development of Bruchidius atrolineatus and Callosobruchus maculatus in Vigna unguiculata in cultures of Nigeria. Insect Science 6: 691-699.
13. Mahdi SHA, Khalequazzaman M (2006) Toxicity studies of some inert dusts with cowpea beetle, Callosobruchus maculatus (F) (Coleoptera: Bruchidae). Journal of Biological Science 6: 402-407.
14. Booker RH (1967) Observation on three bruchids associated with cowpea in Northern Nigeria. Journal of Stored Product Research 3(1): 1-15.
15. Ghosh SK, Dubey SL (2003) Integrated management of insects and stored grain pests. International Book Distributing Co. Lucknow, India, pp. 263.
16. White NDG (1995) Insect mite and insecticides in stored grain ecosystem. In: Stored Grain Ecosystem, Jayas DS, White NDG et al. (Eds.), Marcel Dekker, New York, USA, pp. 123-168.
17. Kumar R, Srivastava M, Dubey NK (2007) Evaluation of Cymbopogon martini oil extract for control of post harvest insect deterioration in cereal and pulse. Journal of Food Protection 70: 172-178.
18. Dubey SC, Suresh M, Singh B (2007) Evaluation of Trichoderma species against Fusarium oxysporum f. sp. Ciceris for integrated management of chickpea wilt. Biological Control 40(1): 118-127.
19. Islam MS, Hasan MM, Xiong W, Zhang SC, Lei CL (2009) Fumigant and repellent activities of essential oil from Coriandrum sativum (L) (Apiaceae) against red flour beetle Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Journal of Pest Science 82(2): 171-177.
20. Anonymous (2000) The Montreal protocol on substances that deplete the ozone layer (with amendments). United Nations Environment Programme, Nairobi, Kenya.
21. Tyler PS, Taylor RW, Rees DP (1983) Insect resistance to phoshine fumigation in food ware houses. Bangladesh International Pest Control 25: 10-13.
22. Collins PJ, Daglish GJ, Bengston M, Lambkin TM, Pavic H (1998) Genetics of resistance to phosphine in Rhyzopertha dominica (Coleoptera: Bostrichidae). Journal of Economic Entomology 91(4): 862-869.
23. Lorini I, Collins PJ, Daglish GJ, Nayak MK, Pavic H (2007) Detection and characterization of strong resistance to phosphine in Brazilian Rhyzopertha dominica (F) (Coleoptera: Bostrychidae). Pest Manag Sci 63(4): 358-364.