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Dual Effect of Plant Materials Against Pulse Beetle *Callosobruchus maculatus* F. (Chrysomelidae: Coleoptera) on Chickpea

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Abstract. The laboratory studies were undertaken to evaluate the repellent effect of different plant materials i.e. lemon, neem, nerium, *Acacia*, *Eucalyptus* at 6% concentration (w/w) against *Callosobruchus maculatus* on chickpea. In addition, the corrected mortality and seed characteristics (seed germination %, root length and seed vigor Index) were also observed. The results showed a repellent effect with an index value of RI=0.8 for lemon and *Eucalyptus* at 24 h. The overall corrected mortality showed the highest (24.73%) at neem and the lowest (4.30%) at *Eucalyptus*. The seed characteristics showed that storage of chickpea seeds until two months was ideal. The best results were recorded after one month of preservation in *Acacia* and control with maximum germination (100%), root length (2.18±0.06 and 3.25±0.11 cm) and V.I (219.0 and 326.0) followed by neem with germination of 96.67% and lemon with root length of 2.37±0.18 cm and V.I 222.13. Thus, it is concluded that all plant materials were not good repellent for pulse beetle, whereas, neem extract displayed an efficient efficacy to kill the pest and lemon showed an ideal repellent effect.

Keywords: pulse beetle, botanical extracts, store grain pest, seed characteristics, *Eucalyptus*

Introduction

Pulses are economically important crops due to their high protein content and good sources of vitamins, iron and calcium (Bhalla *et al*., 2008). These are also practiced as significant rotational crops in many countries of the world which help in soil fertility (Siddique and Sykes, 1997). In Pakistan, these crops are cultivated on an area of more than 1 million hectares with an approximate production of 0.5 ton/ha. This is the only one third production as compare to the developed countries of the world including China (2.4 ton/ha), USA (1.9 ton/ha) and Canada (1.7 ton/ha), respectively (FAO, 2009). On the global basis, chickpea is the third among pulses constituting 88% of the rain-fed cropping method and alone contributes about three fourth of the pulses grown in Pakistan (Ali *et al*., 1991).

The major losses (50 to 60%) in seed weight and protein content occur during storage by store grain pests because in case of their severe attack, the seeds become unfit for human consumption and further planting which results in considerabale economic loss (AIS, 2017; Hamdia *et al*., 2017; Verkaart *et al*., 2017; Sharma and Meshram, 2006). In store grain pests, pulse beetle (*Callosobruchus maculatus*) has been reported as one of the most important insect pests of chickpea. However, it is cosmopolitan in nature and damages number of stored grains including maize, sorghum and cotton seeds (Ahmed *et al*., 2003). The larval stage of beetle is responsible for damaging the seeds by feeding inside the seed kernel. However, the adults play a significant role in increasing breed by oviposit their eggs on grains. Beside their eggs, their excreta cause bad odours and do fermentation those made grains quite poisonous for human as well as for animal consumption. The infestation has been reported higher in summer season as compared to winter season that displays high temperature and relative humidity favour breeding during storage (Jilani and Saxena, 1988). Overall, the pulse beetle is responsible for qualitative and quantitative loss of stored products (Paneru and Shivakoti, 2001).

Since the use of insecticides is not advised directly on food grains due to their residual effect, the different plant extracts have been practiced as grain protectants for last few years as these plant materials are cost-effective, environmental-friendly and easy to apply (Nizamani *et al*., 2020; Saxena and Sayyed, 2018). In this connection, neem and its extracts (oil seed or leaf

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extracts) have been reported as an effective repellent by several researchers against *C. maculatus*. Apart from neem, few other plant materials also tested and reported by (Tripathy *et al*., 2001) their effective results against *C. maculatus* attacking black gram. Al-Lawati *et al.* (2002) tested the potential of eight plant extracts against oviposition, adult emergence and mortality of *C. maculatus*. 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 turmeric) to control pulse beetle. Aslam *et al.* (2004) tested the bio-efficacy of ten plant materials including the leaves of olive, tea, bhang, elephanta, neem, dharek and fruits of garlic, cloves, black pepper and red chilies in powdered form against biology and life span of *C. maculatus*. At present, the pest control measures during storage mainly rely on synthetic insecticides and fumigants. However, the previous studies regarding the use of botanical leaf extracts to repel and kill various insect pests have been proven good results, thus encouraged the further exploration of botanical materials against stroed grain pests. Therefore, the present study has been designed to use botanical plants to observe their effect against *C. maculatus*.

Materials and Methods

Experimental site. The experiment was conducted in post graduate laboratory, Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tando Jam.

Insect collection. The culture of pulse beetle was obtained from the Grain Storage Research Laboratory, University of Karachi, Sindh.

Insect rearing. The obtained culture was reared on chickpea for further multiplication and kept under controlled conditions at 26±3 °C, 12:12 L: D and 75±5% relative humdity.

Plant materials. Five plant species were selected as seed protectants such as neem (*Azadirachta indica*), lemon (*Citrus* sp.), babur (*Acacia nilotica*), bedmushik (*Eucalyptus* sp.) and nerium (*Nerium oleander*) in 2018.

Preparation of plant materials. All the plant materials were brought from agricultural field around Tando Jam. These plant materials were washed, air dried under shade and then pulverized using electric blender (Electric blender, GCG289, China). The blended plant leaves were sieved by muslin cloth to prepare in a proper powder form and later applied on chickpea seeds against pulse beetle.

Experimental outline. The leaf material of each treatment was applied at the rate of 6% (w/w) on 1 Kg chickpea grains. However, the actual grain's weight for experimental purpose was 100 g. The leaves extracts were shaken thoroughly with seed in jars for 3 min to create a homogenous mixture and later kept in plastic jars (11 x 5 inches). The moisture of seeds was checked with the hot oven method as previously described by Sharma and Hanna (1989). The moisture of all selected seeds was kept same to avoid any error during experimentation. A sample of 20 g grains was taken and kept inside the hot oven for 20 sec. Later, the sample was re-weighed and similar practice kept continued until constant moisture gain. Meanwhile, for final moisture calculation we followed the procedure as previously described by Law-Ogbomo and Enobakhare (2007).

Repellence index test. The repellence index (RI) was calculated using the area preference procedure as mentioned by Obeng-Ofori *et al.* (1998). Two small containers having treated and untreated grains kept separately. Both containers were joined with a small pipe or openings as insects could move through it (Fig. 1). Ten insects were randomly selected and released on the treated grains and repellent effect was observed by following formula (Mazzonetto, 2002).

$$RI = 2\ G/G + P$$

Here, G is the percentage of insects present on grains in jar with treated leaf extracts and P is the percentage

Fig. 1. Display model of treated and control seeds kept for repellent test in jars.

of insects present on grains in control jar. The repellency index was observed as if value < 1 shows repellent effect, if value > than 1 shows attractant and if value = 1 shows neutral.

Mortality assessment. In each container (treatment), 20 freshly emerged beetles (10 pairs) were transferred on the treated seeds. These containers were covered with muslin cloth and tighten with an elastic rubber band to avoid any escaping. The corrected mortality of the insects was recorded after 24 h, 48 h and 72 h. The mortality assessment was observed using (Schneider-Orelli, 1947) formula as mentioned below.

$$Pt = \frac{P_o - P_c}{100 - P_c} \times 100$$

where;
Pt=corrected mortality (%); P_o=observed mortality (%) and P_c=mortality (%) in control treatment.

Seed vigor index. In another experiment, 100 g seeds of each treatment was kept separately in a plastic jar and a sample of 10 seeds after one, two and three month intervals were taken to observe vigor index (V.I) of seeds. To check V.I, all the treated seeds were grown on jute bed in a Petri dish (10 x15 mm) inside the laboratory to observe their germination and to measure root length with measuring ruler. The vigor index of seeds was calculated using following formula as given by Abdul-Baki and Anderson (1973).

Vigor index (V.I) = germination percentage x root length (cm)

Statistical analysis. All the data were calculated according to the mentioned formula, meanwhile the data for number of repelled insects and measuring root length were statistically analyzed using analysis of variance (ANOVA) and mean differences were also compared by LSD test at P<0.05 using Statistix software (ver 8.1).

Results and Discussion

Repellent effect of plant materials against pulse beetle *C. maculatus* after 24, 48 and 72 h. The repellent effect of plant materials against pulse beetle after 24, 48 and 72 h was determined statistically (P<0.05) and presented in Table 1. The data showed after 24 h of application the number of insects were significantly higher (6.00±0.57) at T1 lemon and T5 *Eucalyptus* followed by T2 neem (1.66±0.33). Similarly, the index value for 24 h showed repellent effect for T1 lemon and T5 *Eucalyptus* (RI=0.8). However, the rest of the results showed an attractant effect for other treatments.

Table 1. Repellent effect of plant materials against pulse beetle after 24, 48 and 72 h

Treatments	Mean ± S.E	G Value%	P Value%	R Index	Remarks/Effects
24 h					
T1 Lemon	6.00±0.57a	40.00	60.00	0.80	Repellent
T2 Neem	1.66±0.33b	83.33	16.67	1.67	Attractant
T3 Nerium	0.33±0.33b	96.67	3.33	1.93	Attractant
T4 *Acacia*	1.00±0.57b	90.00	10.00	1.80	Attractant
T5 *Eucalyptus*	6.00±0.57a	40.00	60.00	0.80	Repellent
48 h					
T1 Lemon	1.00±0.57bc	75.00	25.00	1.50	Attractant
T2 Neem	0.33±0.33c	96.00	4.00	1.92	Attractant
T3 Nerium	2.67±0.33a	72.41	27.59	1.45	Attractant
T4 *Acacia*	1.67±0.33ab	81.48	18.52	1.63	Attractant
T5 *Eucalyptus*	1.33±0.33bc	66.67	33.33	1.33	Attractant
72 h					
T1 Lemon	1.66±0.33bc	44.44	55.56	0.89	Repellent
T2 Neem	0.00±0.00 d	100.00	0.00	2.00	Attractant
T3 Nerium	4.33±0.33 a	38.10	61.90	0.76	Repellent
T4 *Acacia*	2.33±0.33 b	68.18	31.82	1.36	Attractant
T5 *Eucalyptus*	1.33±0.33 c	50.00	50.00	1.00	Neutral

Means followed by different letters within the same column are significantly different (P<0.05); G represents treated, P represents control and R represents repelled insects.

After 48 h of application, although the higher number of insects (2.67±0.33) was noticed in T3 nerium and the lowest (0.33±0.33) in T2 neem but the repellent index displayed that all treatments were attractant with RI value of more than 1. After 72 h of application, significantly higher number of insects (4.33±0.33) was noted in T3 nerium and no any insect was observed in T2 neem due to the mortality of all insects. Similarly, the index value for 72 h showed repellent effect for T1 lemon (RI=0.89) and T3 nerium (RI=0.76). However, the results showed neutral (RI=1.00) for T5 *Eucalyptus*.

Corrected mortality of *C. maculatus* by botanical materials. The effect of botanical materials on corrected mortality of *C. maculatus* at different time intervals under laboratory conditions is presented in Table 2. The results indicated that maximum value for corrected mortality of *C. maculatus* was observed for T2 neem after 24 h (4.04%), 48 h (10.31%) and 72 h (8.33%) followed by T1 lemon after 24 h (4.04%), 48 h (03.09%) and 72 h (7.29%). The overall highest value for corrected mortality of *C. maculatus* was (24.73%) in T2 neem followed by T1 lemon (16.12%). These results indicated that some plant materials showed their lethal effects late and some showed early.

Vigor index based on germination (%) and root length (cm). The results in Table 3 indicated that when treated seeds were grown and checked after the first month, the maximum germination (100%) was observed in T4 *Acacia* and T6 control, in the second month, the maximum germination (100%) was observed in T6 control followed by T4 *Acacia* (96.67%) and in the third month, the maximum germination (100%) was observed in T6 control followed by T1 lemon (53.33%), respectively. Overall, the results were better for untreated seeds (control treatment) and showed some effect of treatments on seed germination as compared to untreated seeds (Fig. 2).

Table 2. Effect of botanical materials on corrected mortality of *C. maculatus* at different time intervals under laboratory conditions

Treatments	24 h	48 h	72 h	Overall
T1 Lemon	4.04%	3.09%	7.29%	16.12%
T2 Neem	4.04%	10.31%	8.33%	24.73%
T3 Nerium	3.03%	1.03%	1.04%	5.37%
T4 *Acacia*	2.02%	2.06%	5.21%	10.75%
T5 *Eucalyptus*	2.02%	1.03%	1.04%	4.30%

The effect of botanical materials on root length also found significantly different ($P<0.05$). After one month of sowing, the maximum root length (3.25±0.11) was observed in T6 control followed by T1 lemon (2.37±0.18); in the second month, the maximum root length (3.00±0.19) was observed in T6 control followed by T3 nerium (1.90±0.10) and in the third month of sowing, the maximum root length (2.75±0.15 cm) was observed in T6 control followed by T2 neem (1.50±0.15). Accordingly, the vigor index (V.I) based on the results of seed germination percent and root length after the first month showed maximum (326.00) in T6 control followed by T1 lemon (222.13), in the second month, the maximum V.I (300) in T6 control followed by T3 nerium (176.39) and in the third month, the maximum V.I (275) in T6 control followed by T2 neem (69.9).

The application of botanical materials achieved effective results as these materials did not show only mortality of pulse beetle but also indicated efficient repellent effects. The results of the present study showed that the lemon and *Eucalyptus* among used botanical materials

Table 3. Germination %, root length (cm) and seed vigor index of chickpea seed after 1, 2 and 3 months of sowing

Treatments	Germination%	Root length (cm)	Vigor index
1st month			
T1 Lemon	93.33%	2.37±0.18b	222.13
T2 Neem	96.67%	1.97±0.03c	190.44
T3 Nerium	96.67%	1.93±0.13c	186.57
T4 *Acacia*	100.00%	2.18±0.06bc	219.00
T5 *Eucalyptus*	93.33%	1.90±0.03c	177.32
T6 Control	100.00%	3.25±0.11a	326.00
2nd month			
T1 Lemon	86.67%	1.33±0.13cd	115.27
T2 Neem	80.00%	1.72±0.20bc	137.60
T3 Nerium	93.33%	1.90±0.10b	176.39
T4 *Acacia*	96.67%	1.21±0.09d	116.97
T5 *Eucalyptus*	90.00%	1.71±0.13bc	158.40
T6 Control	100.00%	3.00±0.19a	300.00
3rd month			
T1 Lemon	53.33%	0.78±0.13b	41.34
T2 Neem	46.67%	1.50±0.15b	69.9
T3 Nerium	40.00%	1.23±0.28b	48.00
T4 *Acacia*	46.67%	0.99±0.09b	45.54
T5 *Eucalyptus*	40.00%	1.47±0.51b	58.8
T6 Control	100.00%	2.75±0.15a	275

Means followed by different letters within the same column are significantly different ($P<0.05$)

Fig. 2. Measuring root length with ruler and observing germination test (%) of treated chickpea seeds.

were most repellent against pulse beetle after 24 h and similar results were observed for lemon and nerium after 72 h of application. These results are in line with Abdul *et al.* (2017) and Najafzadeh *et al.* (2019) who stated that most plants extracts showed insecticidal action by affecting through mortality, inhibition of adult emergence and acting repellency. Furthermore, Bhuwan and Tripathi (2011) observed the highest repellent activity (90%) for *Schyzygium aromaticum* (essential oil) against *Sitophilus oryzae*. Udo (2011) tested the biological activity of *Zannthoxylum zanthoxyloids* against *Sitophilus zeamais* and *C. maculatus* and found that the extracts also showed moderate repellent effect against the two insect pests. In herbal plants, numbers of different local species have been previously reported as repellent and toxics to *Triticum castaneum* (Suthisut *et al*., 2011).

However, neem did not display the repellent effect in the present study instead the highest mortality effect which did not allow insects to move towards safer (control) place. Khinchi *et al*. (2017) reported that the neem leaf extract at 60 g/Kg grains was found to be highly effective in inhibiting the oviposition (55.40%), decreased eggs hatching (40.88%) and reduced the adult emergence (67.71%) of *C. maculatus*. To study the bioactivity of plant extracts against stored-grain insect pests always generates a renewed interest of various scientists because grains are stored for human survival. Each and every botanical extract has its own effect, meanwhile two effects are common either killing or repellency from the target area. For instance, an application of Eujenol achieved 100% mortality of *T. castaneum*, however, similar insects (*T. castaneum*) were repelled by application of *Bactris campestris* and *Veronica arvensis* (Zia *et al*., 2011). Basically, the efficacy of the botanical leaf materials may arise from prejudicing respiration through ceasing of insect spiracles which results in suffocation and death of insect (Dales, 1996). In such cases, these botanical materials that possess much efficacy or fumigantant like neem and lemon could damage more as compared to other plant materials having less vapor density.

Conclusion

It is concluded that the lemon was found most repellent against pulse beetle and the highest mortality was observed by neem. Maximum germination, root length and vigor index were found better upto the second month of sowing in all treatments.

Conflict of Intrest. There is no conflict of interest among all authors

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