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Repellent Effect of Ethanol Extracts of Plant Materials on *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

Javed Iqbal*, Abdul Qayyum and Syed Zahid Mustafa
Planning & Research Monitoring Cell (JI), Institute of Agricultural Biotechnology and Genetic Resources (AQ) and Directorate of Administration & General Services (SZM)

Abstract.- Out of seven plant species tested, ethanol extract of sweet flag (*Acorus calamus)* and neem (*Azadirachta indica*) exhibited more than 40% average repellency over 8 weeks against red flour beetle at all application rates. Turmeric (*Curcuma longa*) and harmal (*Peganum harmala*) showed this level at 1600 μg/cm^2 and 800 μg/cm^2 only whereas kuth (*Saussurea lappa*) showed promising repellency only at 1600 μg/cm^2. Repellency was considerably higher during 1st week of testing. Neem was comparatively persistent as it remained effective throughout 8 weeks although showing relatively lower repellency than that of sweet flag in the beginning.

Key words: Repellency, botanical pesticides, *Azadirachta indica*, *Acorus calamus*, *Curcuma longa*, *Valariana officianalis*, *Peganum harmala*, *Saussurea lappa, Skimia laureola*, *Tribolium castaneum*

INTRODUCTION

Stored grains in Pakistan suffer colossal loss both in quantity and quality due to insect pests. Major stored grain insect pests in Pakistan include *Tribolium castaneum* (Herbst) (Red flour beetle), *Rhizopertha dominica* (F.) (Lesser grain borer), *Sitophilus oryzae* (L.) (Rice weevil), *Trogoderma granarium* Everts (Khapra beetle), and *Sitotroga cerealella* (Oliver) (Angoumois grain moth). Control of these insect pests is primarily dependant upon continuous application of insecticides and fumigants. Excessive use of pesticides has given rise to many well known and serious problems to the environment and the development of resistance (Champ and Dye, 1977; Irshad and Gillani, 1989, 1991; Irshad *et al.,* 1992; Irshad and Iqbal, 1994). The problems caused by pesticides and their residues have increased the need for effective biodegradable pesticides with greater selectivity. Essential oils having compounds monoterpenoids, offer promising alternatives to classical fumigants (Huang *et al*., 2000; Papachristos and Stamopolos, 2002, 2003), contact insecticides (Saxena *et al*., 1992; Weaver *et al*., 1994), repellents (Saim and Meloan, 1986; Ndungu *et al*., 1995), antifeedants (Harwood *et al*., 1990; Chiam *et al*., 1999) and may also affect some biological parameters such as growth rate, life span and reproduction (Pascual-Villalobos, 1996).

Plants provide potential alternatives to currently used insect control agents. In the past, few indigenous plants of Pakistan were studied for repellent effects on red flour beetle (Jilani *et al.,* 1989, 1991, 1993) and for their repellent and feeding deterrent effects against lesser grain borer (Jilani and Saxena, 1990). Chemicals that prevent insect damage to plants or animals by rendering them unattractive or offensive are called repellents. There are two broad types of repellents. Physical repellents produce repellency by physical means and are of contact stimuli, auditory, barrier, visual, excitatory and anti-feeding actions. Chemical repellents affect tectile, olfactory or gustatory receptors of insects and could be of plant origin or synthetic (Mahulikar and Chavan, 2007).

The present investigation has been carried out with a view to assess the chemical repellent effect of plant species viz. neem (*Azadirachta indica)*, sweet flag (*Acorus calamus*), turmeric (*Curcuma longa*), balchar (*Valariana officianalis)*, harmal (*Peganum harmala),* kuth (*Sausarea lappa*) and ner (*Skimia laureola)* against storage pests. These plants are aboriginal, abundantly available and possess medicinal properties. Some of these plants have also been found effective as repellent and growth inhibitor against *T. castaneum* (Kanvil *et al*., 2006)

* Corresponding author: linkjaved@gmail.com
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and peach fruit fly, *Bactrocera zonata* (Jilani *et al*., 2006; Rehman *et al*., 2009).

MATERIALS AND METHODS

T. castaneum was reared in the laboratory on wheat flour media (fine flour:corn flour: Brewer's yeast in the ratio of 7:2:1) under controlled temperature 28±1°C and 65±5% relative humidity in the laboratory of Insect Pest Management Program, National Agricultural Research Centre, Islamabad. The full-grown plant materials {neem (seeds), sweet flag (rhizomes), turmeric (rhizomes), balchar (roots), harmal (seeds), kuth (roots) and ner (leaves)} were purchased from the local market and were grounded to fine powder of 60 mesh. The extracts were obtained separately on Soxhlet's extractor with ethanol for 8 hours. The extracts were concentrated on rotary evaporator and finally made solvent free in a vacuum desiccator. Final extracts were used to prepare stock solution in acetone and diluted to desired concentration.

Repellency test was performed by paper strip method described by McDonald *et al.* (1970) and used by Jilani and Su (1983) in a dark place with temperature at 28±1°C and relative humidity 65±5%. Strips of Whatman filter paper No. 1 measuring 10x5 cm were treated separately with extracts of different plant materials to get the deposits of 1600, 800 and 400 μg/cm^2. Acetone treated strips served as untreated. After the evaporation of acetone, extract treated strips were attached lengthwise, edge-to-edge to untreated strips of similar size with cellophane tape on the reverse side. Each concentration had four replications and each paper was rotated clockwise, hence direction of each strip was changed to minimize the effects of light and adjacent strips, if any. A glass ring measuring 2.5 cm high with 7 cm internal diameter was placed over the two matched strips so that the joined edges bisected the ring. Ten days old adults starved for 24 hours were used each time and no food was provided to insects through out the experiment period. Ten adults were released in the middle of the test arena. Insects settled on treated and untreated halves were counted daily at 09:00 and 15:00 hours for five consecutive days. Percent repellency was calculated as under.

$$\text{Percent repellency} = \frac{\text{No. of adults on untreated half} - \text{No. of adults on treated half}}{\text{Total No of adults released}} \times 100$$

Fresh test insects were introduced in 2nd, 4th and 8th week after treatment, using same treated papers. Weekly repellency, persistence up to 8 weeks and overall average repellency of different treatments was compared. Each treatment was replicated four times. Experiment was designed in Randomized Complete Block Design (RCBD) and each replication served as block. Statistical difference, significance and interaction of various treatments were calculated using computer program MSTAT-C.

RESULTS AND DISCUSSION

During 1st week highest repellency of 90% was observed in sweet flag at 1600 μg/cm^2 application rate followed by 88.50, 85.00, 79.25, 74.00, 69.50 and 21.00 in turmeric, neem, harmal, balchar, kuth and ner respectively (Table 1). In all the plants repellency values were statistically non-significant from each other except ner. A comparison of 8 weeks average repellency showed that sweet flag at 1600 μg/cm^2 had the highest repellency of 75.38% followed by neem, harmal, turmeric, kuth, balchar and ner respectively.

During 2nd week sweet flag remained the highest repellant followed by neem, harmal and kuth. All were non-significant at comparable application rates. During 4th and 8th week sweet flag maintained its effectiveness at 1600 μg/cm^2 but at lower application rates it decreased from 77.50 to 42%. Similar trend was also observed in kuth and harmal. Neem was comparatively persistent as it remained effective through out the 8 weeks although showing comparatively lower repellency than that of sweet flag (Table I).

Accumulated data indicated that Neem had the highest average repellency of 68.69% followed by sweet flag, harmal, kuth, turmericner and balchar having 64.56, 51.10, 40.50, 40.02, 24.04 and 20.96%, respectively (Fig. 1). Both neem and sweet flag were non-significant from each other however, there was a significantly difference from rest of the plants.

Table I.- **Repellency of ethanol extracts of plants to *T. castaneum* adults at different time interval with a choice of treated and untreated filter paper.**

Plant	Application Rate (μg/cm²)	Average per cent Repellency at indicated week after treatment									
		1st		2nd		4th		8th		Average	
Neem	1600	85.00	a	79.50	a	70.50	a	65.00	a	75.00	a
	800	75.00	abc	71.00	ab	67.50	a	58.00	ab	67.88	a
	400	62.75	abc	73.75	ab	59.50	ab	56.75	abc	63.20	ab
Sweet flag	1600	90.00	a	81.00	a	72.50	a	58.00	ab	75.38	a
	800	86.00	a	77.50	a	42.00	a-d	42.00	a-d	61.88	ab
	400	75.00	abc	72.00	ab	40.00	a-d	38.75	a-d	56.45	abc
Turmeric	1600	88.50	a	74.50	ab	34.50	a-e	23.50	b-e	55.25	a-d
	800	80.50	a	38.00	a-f	27.00	a-e	19.50	b-e	41.25	b-e
	400	42.00	b-e	13.75	c-g	20.50	b-e	18.00	cde	23.58	efg
Balchar	1600	74.00	abc	43.00	a-e	-9.50	e	12.00	de	29.88	def
	800	62.50	abc	16.00	g	15.50	b-e	17.50	cd	19.88	efg
	400	7.00	ef	3.00	efg	-2.00	de	44.50	a-d	13.13	fg
Harmal	1600	79.25	ab	64.50	ab	56.00	abc	37.50	a-d	59.33	ab
	800	72.50	abc	57.00	abc	47.00	abc	31.50	a-d	52.00	a-d
	400	68.00	abc	53.00	a-d	19.50	b-e	27.50	a-d	42.00	b-e
Kuth	1600	69.50	abc	61.50	ab	34.50	a-e	33.00	a-d	49.63	a-d
	800	52.00	a-d	28.00	b-g	13.00	cde	32.00	a-d	31.25	c-f
	400	60.25	abc	46.00	a-e	28.00	a-e	25.50	a-e	39.93	b-e
Ner	1600	21.00	def	0.00	efg	34.50	a-e	39.50	a-d	23.75	efg
	800	37.00	c-f	-5.00	fg	33.00	a-e	31.00	a-d	24.00	efg
	400	25.00	def	8.00	d-g	34.50	a-e	30.00	a-d	24.38	efg
Control		4.75	f	12.50	c-g	-6.00	e	11.00	e	0.00	g

Each value is mean of 4 replications; 10 adults per replication.
Values having same letters in a column are non-significant ($P \leq 0.05$); DMRT, Duncan, 1951.

Comparison of repellency of different application rates revealed that repellency of plants was dose dependant. At 1600 μg/cm² repellency was 52.60% which was significantly higher than 42.59% at 800 μg/cm² and 37.62% at 400 μg/cm² (Fig. 2). Statistically application rates of 800 and 400 μg/cm² were non-significant.

Comparison of repellency in different weeks revealed that average mean repellency of plant extracts during first week being 62.55% was the highest and significantly different from rest of the weeks. Average repellency during second week being 44.02% was significantly higher than fourth and eighth week. However, during 4th and 8th week repellency values of 35.19 and 35.31%, respectively, were not significantly different. This indicated a significant decrease in repellency during second and fourth week but later on decrease was non-significant (Fig. 3).

Interaction of application rates and time weeks presented in Table II revealed that 1600 μg/cm² application rate interaction was effective through out the experimental period except week 8. During 1st week at all application rates repellency values were statistically significant from the remaining values at the corresponding application rates. Highest repellency was 72.46 in 1st week and 57.71 during 2nd week. Both the values were significantly different from each other. However, values of 4th and 8th week were statistically non-significant. At 800 μg/cm^2 values of 1st week were significantly different from 2nd, 4th and 8th week.

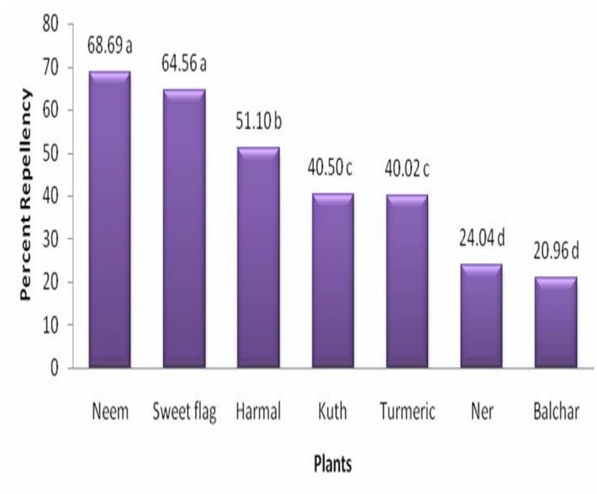

Fig. 1. Mean percent repellency of various plants to *T. castaneum.*
Average of 3 application rates; 4 weeks and 4 replications; 10 adults per replication; Values having same letters are non-significant (P≤0.05); DMRT, Duncan, 1951

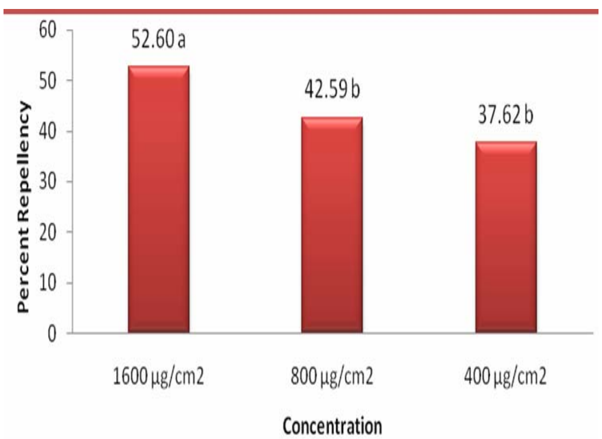

Fig. 2. Mean percent repellency of various application rates to *T. castaneum*
Average of Average of 7 plants; 4 weeks and 4 replications; 10 adults per replication; Values having same letters are non-significant (P ≤ 0.05); DMRT, Duncan, 1951

Data in Table III show the effect of plants and weeks interaction. Average repellency during 1st week of all the plants was higher in all cases except ner. The repellency decreased gradually. The plants

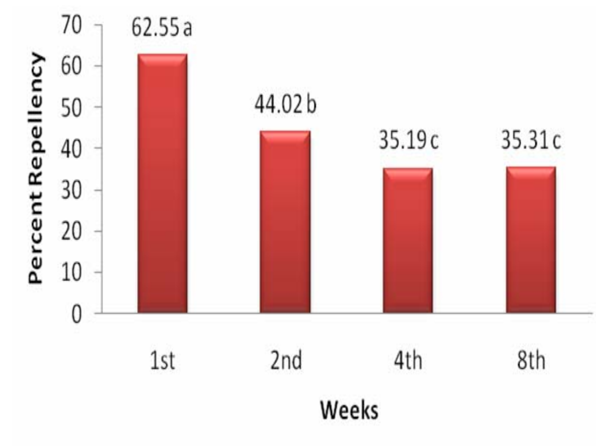

Fig. 3. Mean percent repellency after various weeks to *T. castaneum.*
Average of 7 plants; 3 application rates and 4 replications; 10 adults per replication; Values having same letters are non-significant (P≤0.05); DMRT, Duncan, 1951

Table II.- Interaction of percent repellency of various application rates and weeks to *T. castaneum.*

Application rates (µg/cm^2)	Average percent repellency after weeks							
	1st		2nd		4th		8th	
1600	72.46	a	57.71	bc	41.86	de	38.36	de
800	66.50	ab	35.79	de	35.00	de	33.07	de
400	48.68	cd	38.57	de	28.71	e	34.50	de

Average of 7 plants and 4 replications; 10 adults per replication; Values having same letters in columns and rows are non-significant (P ≤ 0.05); DMRT, Duncan, 1951

Table III.- Interaction of percent repellency of various plants and weeks to *T. castaneum.*

Plant	Average percent repellency after weeks			
	1st	2nd	4th	8th
Neem	74.25abc	74.75 abc	65.83a-e	59.92a
Sweet flag	83.67a	76.83ab	51.50c-h	46.25f
Turmeric	70.33a-d	42.08e-j	27.33h-k	20.33d-f
Balchar	47.83d-i	10.00kl	1.33hl	24.67ikl
Harmal	73.25abc	58.17b-g	40.83f-j	32.17h-k
Kuth	60.83a-f	45.30e-i	25.50ijk	30.33h-k
Ner	27.67h-k	1.00l	34.00g-k	33.50h-k

Average of 3 application rates; and 4 replications; 10 adults per replication; Values having same letters in columns and rows are non-significant (P ≤ 0.05); DMRT, Duncan, 1951

possessing more than 40% value decreased their effectiveness during 4th and 8th week except neem and sweet flag. During the 1st week, although neem had lower value than sweet flag (74.25 and 83.67, respectively) yet it was more persistent as the values in 8th week was 59.92 and 46.25%, respectively. Harmal was next to sweet flag which had 40.83% repellency during 4th week which decreased to 32.17% during 8th week. Turmeric showed repellant effect upto two weeks as the repellency during 4th week decreased to 27.33%. It was 42.08% during 2nd week.

In Pakistan, previously *Astagalus anisacanthus*, *Curcuma zedoaria*, *Ephedra intermedia*, *Ferula assafoetida*, *Foeniculum graecium*, *Nerium indicum*, *Salsola kali*, *Sophora griffuhii*, have been screened against *T. castaneum* and maximum average repellency of 57.6 have been recorded in *A. anisacanthus* (Jilani *et al*., 1991). This value is much less than the repellency recorded in neem, sweet flag and turmeric in this study. In another experiment, *Agriophylum latifolium*, *Ephedra* sp., *Ferula oopoda*, *Baloxylon griffithii*, *Hertia intermedia*, *Malva neglecta*, *Salvia cabulica*, *S. macrosiphon*, *Saxifraga ciliate* and *Stocksia brahuica* were screened with highest repellency of 45.2 in *S. brahuica* (Jilani *et al*., 1993). Over here also repellency is much less than test plants of this investigation. There is another study by Jilani *et al.* (1989) in which *Achillea millefolium*, *Andrachne trifoliate*, *Berberis aristata*, *Canabis sativa*, *Euphuorbia* spp., *Limonium cabulicum*, *Mentha spicata*, *Neslia apiculata* and *Thymus serphylum* were tested. The highest average repellency of 57.7 was observed in *L. cabulicum*. This is also much lower than neem and sweet flag. In this experiment repellency of balchar, harmal, kuth and ner was almost comparable to the previously tested plants. *T. castaneum* repellency in n-hexane extracted neem is 57, 65, 70 at 200, 400, 800 $\mu g/cm^2$ respectively (Jilani *et al*., 1993) while it is 75, 67, 63 at 1600, 800, 400 $\mu g/cm^2$, respectively Almost same trend is exhibited by sweet flag and turmeric. Thus this study confirms the previous findings that neem, sweet flag and turmeric are better repellent. In some cases the values are negative, indicating attraction. However, it needs further investigations that if these concentrations can be used as attractant.

All these plants are used for medicinal purposes and some are used as spices in daily food in Pakistan and in some other countries. Being natural of such traditional use for prolonged periods they are not likely to leave harmful residues. They should be good sources to use for further development as protectant for cereal grains.

The tested plants are abundantly available in Pakistan. Neem has been traditionally used for protection of stored products since centuries. The promising plant extracts containing active fractions display novel mode of action having effects on egg hatching and adult mortality. Hence these studies provide basis for pest control technology. Such materials can be applied to grains/ bags and can very well be used as a part of IPM of stored grains.

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REFERENCES

CHAMP, B.R. AND DYE, C.E., 1977. FAO global survey of pesticide susceptibility of stored grain pests. *FAO Pl. Prot. Bull*., **25**: 49-67.

CHIAM, W.Y., HUANG, Y., CHEN, S.X. AND HO, H.S., 1999. Toxic and antifeedant effects of allyl disulfide on *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae). *J. econ. Ent.,* **92**: 239-245.

DUNCAN, D.B., 1951. A significance test for differences between ranked treatments in analysis of variance. *Va. J. Sci*., **2**: 171 – 189.

HARWOOD, S.H., MOLDENKE, A.F. AND BERRY, R.E., 1990. Toxicity ofmonoterpenes to the variegated cutworm (Lepidoptera: Noctuidae). *J. econ. Ent.,* **83**: 1761-1767.

HUANG, Y., CHEN, S.X. AND HO, S.H., 2000. Bioactivities of methyl and allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. econ. Ent*., **93**: 537-543.

IRSHAD, M**.** AND GILLANI, W.A., 1989. Resistance in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to malathion in Pakistan. *Pakistan J. Zool*., **22**: 257 -261.

IRSHAD, M**.** AND GILLANI, W.A., 991. Malathion resistance in

Sitophilus oryzae (L) (Coleoptera: Curculionidae) infesting stored grains in Pakistan. *Pakistan J. agric. Res*., **13**: 273 - 276.

IRSHAD, M. AND IQBAL, J., 1994. Phosphine resistance in important stored grain insect pests in Pakistan. *Pakistan J. Zool*., **26**: 347 -350.

IRSHAD, M**.**, GILLANI, W.A. AND IQBAL, J., 1992. Occurrence of resistance in stored grain pests to pesticides in Pakistan. *Pakistan J. Zool*., **24**: 79 -82.

JILANI, G. AND SAXENA, R.C., 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and a neem-based insecticide against Lesser Grain Borer (Coleoptera: Bostrychidae). *J. econ. Ent*., **83**: 629–634.

JILANI, G. AND SU, H.C.F., 1983. Laboratory studies on several plant materials as insect repellents for protection of cereal grains. *J. econ. Ent*., **76**: 154 – 157.

JILANI, G., KHATTAK, M.K. AND SHAHZAD, F., 2006. Toxic and growth regulating effect of ethanol extract and petroleum ether extract of *Valariana officianalis* L. against *Bactrocera zonata* Saunder. *Pak. Ent*., **28**: 11-14.

JILANI, G., ULLAH, N., GHIASUDDIN AND KHAN, M.I., 1991. Repellency of some plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)-II. *Pak. Ent*., **13**: 5 -8.

JILANI, G., ULLAH, N., GHIASUDDIN AND KHAN, M.I., 1993. Repellency of some plant extracts against *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae):V. *Pak. Ent*., **15**: 103-105.

JILANI, G., ULLAH, N. AND GHIASUDDIN, 1989. Repellency of some plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Pak. Entomol*., **11**: 18-22.

KANVIL, S., JILANI, G. AND REHMAN, J.U., 2006. Repellency of petroleum ether extract of some indigenous plants against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Pakistan J. Zool*., **38**: 233-238.

MAHULIKAR, P.P. AND CHAVAN, K.M., 2007. *Botanicals as ecofriendly pesticides*. New India Publishing Agency, New Delhi, India, 264 pp.

MCDONALD, L.L., GUY, R.H. AND SPEIRS, R.D., 1970. *Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects 1*. USDA Marketing Respectively. Report, 882 pp.

NDUNGU, M., LAWNDALE, W., HASSANALI, A., MOREKA, L. AND CHHABRA, S.C., 1995. *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. *Entomologia Experimentalis et Applicata,* **76**: 217-222.

PAPACHRISTOS, D.P. AND STAMOPOULOS, D.C., 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J. Stored Prod. Res*., **38**: 117-128.

PAPACHRISTOS, D.P. AND STAMOPOULOS, D.C., 2003. Selection of *Acanthoscelides obtectus* (Say) for resistance to lavender essential oil vapour. *J. Stored Prod. Res*., **39**: 433-441.

PASCUAL-VILLALOBOS, J.M., 1996. Evaluation of the insecticidal activity of *Chrysanthemum coronarium* L. plant extracts. *Boletin de Sanidad Vegetal Plagas*, **22**: 411-420.

REHMAN, J. U., JILANI, G., KHAN, M.A., MASIH, R. AND KANVIL, S., 2009. Repellent and oviposition deterrent effects of indigenous plant extracts to Peach Fruit Fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae). *Pakistan J. Zool*., **41**: 101-108.

SAIM, N. AND MELOAN, C.E., 1986. Compounds from leaves of bay *(Laurus nobilis* L.) as repellents for *Tribolium castaneum* (Herbst) when added to wheat flour. *J. Stored Prod. Res*., **22**: 141-144.

SAXENA, R.C., DIXIT, D.P. AND HARSHAN, V., 1992. Insecticidal action of *Lantana camara* against *Callosobruchus chinensis* (Coleoptera: Bruchidae). *J. Stored Prod. Res*., **28**: 279-281.

WEAVER, D.K., DUNKEL, F.V., POTTER, R.C. AND NTEZURUBANZA, L., 1994. Contact and fumigant efficacy of powdered and intact *Ocimum canum* Sims (Lamiales: Lamiaceae) against *Zabrotes subfasciatus* (Boheman) adults (Coleoptera: Bruchidae). *J. Stored Prod. Res*., **30**: 243-252.

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