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Insecticidal and Repellent Potential of Citrus Essential Oils Against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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Abstract.- In this study citrus oils extracted from citrus seeds were tested on different growth stages of *T. castaneum*, an important pest of stored grains. On adults LC_{50} of different citrus seed oils were 5.47%, 7.70%, 10.79% and 11.79% for rough lemon (*Citrus jambhiri*), freutrall early (*Citrus reticulate*), kinnow (*Citrus reticulate*) and red blood orange (*Citrus sinensis*), respectively. While for grubs, LC_{50s} were 11.27%, 17.31%, 106.85% and 111.20% for *C. jambhiri*, *C. reticulate*, *C. sinensis* and *C. reticulate*, respectively. Rough lemon (*C. jambhiri*) showed lowest LT_{50} of 6.89 h and 4.06 h against adults and grubs, respectfully. In case of repellency, *C. jambhiri* gave lowest RD_{50s} value (4.40%) against adults when compared with other citrus cultivars. The presence of limonoids in citrus extracts is therefore a significant indicator for pest control that needs to be exploited further.

Keywords: *Tribolium castaneum*, citrus oils, plant extracts.

INTRODUCTION

Stored products of agriculture are attacked by more than 600 species of beetle pests all over the world (Rajendran, 2002). Among beetles, *Tribolium castaneum* is most common and prevalent pest species of stored grains (Zettler and Cuperus, 1990) largely found in warehouses, granaries and mills causing quantitative and qualitative losses (Zettler, 1991; Mondal and Khalequzzaman, 2006). Conventionally it was controlled in past through synthetic insecticides. However main problems with the use of these insecticides are water and soil contamination, build up of resistance and toxicity to non-target species (Jembere *et al.*, 1995; Donahay *et al.*, 1992).

Currently use of Phosphine is the most common method to control these insects worldwide but at the same time problem of increased resistance resulted in control failures in many countries (Collins *et al*., 2002). In order to resolve pesticide dilemma researchers are continuously evaluating botanicals as being eco friendly and highly degradable (Berenbaum, 1989; Grainge *et al.*, 1986). The bio-pesticides have showed acute biocidal activity against various insect species (Tsao *et al*., 1995, Boussalis *et al*., 1999; Abubakar *et al*., 2000). Many plant products have been evaluated for their toxic properties against different stored grain pest insects (Su, 1990; Mukherjee and Joseph, 2000), especially in the form of essential oils which have recently received much attention due to their multi-functions as antimicrobial, antifungal, antitumor and insecticidal agents (De Souza *et al*., 2005; Shaaya *et al.*, 1991).

The essential oils of many plants have repellent and insecticidal activities against pest insects (Ahmed *et al.*, 1988). Besides crude oils, toxic effects of oil constituents have also been observed against many pest insects (Weaver *et al.*, 1991; 1995). Plant extracts contain compounds having ovicidal, repellent, antifeedant, sterilizing and toxic effects on insects (Isman, 2006). The essential oils of several spices like anise (*Pimpinella anisum* L.) and peppermint (*Mentha piperita* L.) have been confirmed to have fumigant toxicity to *T. castaneum* (Herbst). Shaaya *et al.* (1991) and Ho *et al*. (1996) reported that essential oil of garlic has insecticidal effect to *T. castaneum*.

Due to rapid increase in development of resistance in stored product pests against synthetic chemicals, there is an urgent need to develop

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environment friendly alternatives to chemical control. In this attempt, present study was planned to evaluate biopotenial of citrus seed extracts against *T. castaneum.*

MATERIALS AND METHODS

Rearing of insects

To get homogenous population, pairs of *T. castaneum* were released in dark jars and placed in incubator at 30±2°C and 60±5% R.H. Sexing of released insects was done following Halstead (1963). Whole meal wheat and wheat flour sterilized at 60°C for 60-90 min. was provided as culture medium. Mouth of jars was covered with muslin cloth, tied with rubber bands to avoid escape of beetles and for entry of any other insects from outside (Zia *et al.,* 2011). Beetles were allowed to remain in the culture medium for 3 days for egg laying and then removed from jars with the help of sieve # 20 and fine camel hair brushes. The flour containing eggs was again placed in the same jars. Population received from these jars after a month was considered as a homogeneous culture and was used in the experiment.

Citrus collection

Different fruit cultivars of citrus *i.e.,* kinnow (*Citrus reticulate* var kinnow), freutrall (*Citrus reticulate* var freutrall), red blood orange (*Citrus sinensis*) and rough lemon (*Citrus jambhiri*) were collected from Khanpur city (33° 49' North, 72° 55' East) of Khyber Pakhtunkhawa province which were identified from Institute of Horticulture, University of Agriculture, Faisalabad, Pakistan.

Extraction of essential oil

The seeds of citrus cultivars were initially washed with tap water to remove pulp and then dried in oven for 48 h at 60°C and thereafter grinded using an electric grinder. The ground material was placed in Soxhlet apparatus for extraction of oil by steam distillation method (Vogel, 1978). Oil was collected in small vials. Stock solutions were prepared by adding 1ml of oil from each variety in 99ml of acetone and were considered as 1% stock solution from which series of concentration were prepared (Murgan *et al.,* 2007).

Bioassay

Each experiment was carried out using 90mm Petri dish and same for filter paper. Different concentrations of oil (2, 4, 6, 8 and 10%) were applied on the filter paper and it was allowed to dry. Twenty adults were introduced in each covered Petri dish having same food. Mortality rate of adults was recorded after 7 days of exposure to treatments. Same procedure was carried out for grubs but mortality data was recorded after 24, 48 and 72 h of exposure to treatments following Upadhyay and Gayatri (2007).

The experimental method as described by Jilani *et al.* (1990) and Upadhyay and Gayatri (2007) was used with some minor modifications. Whatman No. 1 filter papers (diameter 9cm) were cut in half. Test solutions were prepared by dissolving 1ml of oil in 99ml of acetone to make solution in percentage. Each solution (as discussed above) was applied to half cut filter-paper in a glass disc uniformly with a micropipette. Other half of the filter paper was treated only with acetone as a control. The treated and control half discs were dried thereafter. Treated and untreated halves were then attached to their opposite sides using adhesive tape and placed in Petri dishes. Twenty adult (7–9 day old) beetles of mixed sex (with equal ratio) were released at the centre of each filter paper disc. The dishes were then covered and placed in dark. Three replications were used for each concentration. Observations on the number of insects present on both treated and untreated halves were recorded after 24 h.

Data analysis

Abbot's formula (Abbot, 1925) was used for corrected mortality and data obtained was analyzed by probit analysis (Finney, 1989) by using Minitab-15 software for dose and time mortality regression lines. In control treatment, if mortality rates were between 5 to 20% then percentage mortality was corrected by Abbot's formula as follows:

$$\% \text{ Corrected mortality} = \frac{\% \text{ Observed mortality} - \% \text{ Control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Table I.- Repellent effect of citrus cultivars against red flour beetle adult (*Tribolium castaneum*).

Citrus cultivars	RD_{50} (95%FL)	Slope±SE	χ^2	*P*	Reg. equation
Kinnow seed (*Citrus reticulate*)	8.14 (7.34-13.11)	1.61±0.23	3.07	0.21	Y=1.61X-3.38
Freutrall early (*Citrus reticulate*)	4.41 (3.92-4.78)	2.34±0.27	3.42	0.18	Y=2.34X-3.47
Red blood orange seed (*Citrus sinensis*)	4.84 (4.24-5.33)	1.74±0.23	16.94	0.00	Y=1.74X-2.75
Rough lemon seed (*Citrus jambhiri*)	4.40 (3.96-4.78)	2.42±0.28	2.53	0.28	Y=2.42X-3.60

RESULTS

Adult mortality and lethal time

With regards to LC_{50} for *T. castaneum* adults, rough lemon (*C. jambhiri*) proved promising treatment after 7 days of exposure by having least LC_{50} value 5.47%, followed by freutrall early (*C. raticulata* var freutrall) 7.70%. The red blood orange (*C. sinensis*) was however less effective in terms of LC_{50} *i.e.*, 11.79%, followed by kinnow (*C. reticulate* var kinnow) 10.79% as shown in Table I.

The most effective citrus cultivars against adults was rough lemon (*C. jambhiri*) with 6.89 h LT_{50} value, followed by kinnow (*C. reticulate* var kinnow) with 8.58 h while red blood orange (*C. sinensis*) was less effective with 10.07 h LT_{50} value, followed by 8.81 h for freutrall early (*C. raticulate* var freutrall) as shown in Table II.

Grub mortality and lethal time

On grubs, effective citrus cultivar was again rough lemon (*C. jambhiri*) having lowest LC_{50} value (11.27%) after 3 days of exposure followed by freutrall early (*C. raticulata* var freutrall) 17.32% while kinnow (*C. reticulate* var kinnow) showed highest LC_{50} 111.20% followed by red blood orange (*C. sinensis*) 106.85% as shown in Table I.

The most effective citrus cultivar against grubs was rough lemon (*C. jambhiri*) that took shortest time to kill 50% population (4.06 h), followed by red blood orange (*C. sinensis*) 6.75 h while kinnow (*C. reticulate* var kinnow) and freutrall early (*C. raticulata* var freutrall) took 9.29 and 16 h, respectively to kill 50% population as shown in Table I.

Repellence

In terms of repellency of Citrus cultivars against adults the most effective cultivar was rough lemon (*C. jambhiri*) with RD_{50} value (4.40%), followed by freutrall early (*C. raticulata* var freutrall) 4.41% while kinnow had the highest RD_{50} value (8.14%), followed by red blood orange (*C. sinensis*) 4.84% as shown in Table II.

DISCUSSION

In view of environmental hazards and development of insecticide resistance in insects, recent trend is to explore eco-friendly plant extracts with the potential to suppress pest populations. Although several plant based insecticides have been reported worldwide, there is still a wide scope to investigate more potent plant products with enhanced activity, eco-friendly that could replace objectionable synthetic chemicals. For management of insect pest many plant products such as essential oils and plant extracts have been screened for their mortality and repellent activities (Malik and Naqvi, 1984). Plant extracts are safer for non target organisms (Akram *et al.*, 2010; Din *et al.*, 2011) and are thus more feasible from environmental perspective. Results of present study are in agreement with previous works done evaluating different plants extracts against stored insect pests. Jilani *et al.* (1991) studied the effect of ten-plant materials against red flour beetle *(T. castaneum*). Among, these N-hexane extracts of *Astragalus anisacanthus* (Boiss.), *Foeniculum graecium* (Miller) leaves and flowers of *Sophora griffithi* (Stocks) exhibited more than 40% repellency against adults of red flour beetle. In another study, Zahid *et al.* (2000) made a comparison of neem oil solution with malathion and actellic in relation to their efficacy against flour beetles, *T. castaneum* (H) and *T. confusum* (DUV.). Among these actellic exhibited 100% mortality within 3 days after application whereas Malathion showed 100%

Table II.- Insecticidal activity and time mortality of citrus cultivar against *Tribolium castaneum*.

S.#	Cit. cultivars	Adult					Grub				
		LC_{50}	Slope ±S.E	X^2	P	Reg. eq	LC_{50}	Slope±S.E	X^2	P	Reg. eq
Insecticidal activity											
1	Kinnow Seed (*Citrus reticulate*)	10.79 (8.26-31.31)	0.61±0.21	0.3	0.8	Y=0.6X-1.4	111.20 (102.1-281)	0.36±0.25	0.27	0.8	Y=0.3X-1.7
2	Freutrall early (*Citrus reticulate*)	7.70 (6.16-11.77)	0.63±0.20	1.8	0.4	Y=0.6X-1.3	17.31 (10.82-1156)	0.52±0.21	0.17	0.9	Y=0.7X-1.5
3	Red blood orange (*Citrus sinensis*)	11.79 (8.73-48.60)	0.59±0.21	0.2	0.9	Y=0.5X-1.4	106.85 (98.2-221.1)	0.10±0.20	2.61	0.2	Y=0.1X-0.5
4	Rough lemon Seed (*Citrus jambhiri*)	5.47 (3.11-6.94)	0.59±0.20	0.4	0.7	Y=0.6X-1.3	11.27 (8.80-24.12)	0.73±0.21	1.28	0.5	Y=0.7X-1.7
Time mortality											
1	Kinnow Seed (*Citrus reticulate*)	8.58 (7.89-9.54)	1.35±0.09	3.47	0.6	Y=1.3X-2.9	9.29 (6.31-20.18)	0.89±0.15	0.17	0.6	Y=0.8X-1.9
2	Freutrall early (*Citrus reticulate*)	8.81 (7.47-11.02)	0.52±0.04	2.57	0.7	Y=0.5X-1.1	16 (7.1-281.36)	0.34±0.01	4.96	0.0	Y=0.3X-0.9
3	Red blood orange (*Citrus sinensis*)	10.07 (9.02-11.63)	1.21±0.09	12.5	0.0	Y=1.2X-2.8	6.75 (4.48-17.57)	0.45±0.09	0.04	0.8	Y=0.4X-0.8
4	Rough lemon Seed (*Citrus jambhiri*)	6.89 (6.38-7.55)	1.01±0.06	8.32	0.1	Y=1.0X-1.9	4.06 (3.55-4.93)	1.51±0.12	3.17	0.0	Y=1.5X-1.6

mortality of the test insects within 7 days. The neem oil solution gave 63% mortality within 9 days. In a study by Magdy and Abdelgaleil (2008) essential oils of few plant species showed promising insecticidal activity against *T. castaneum*. Epidi and Odili, (2009) evaluated efficacy of powders of different plants against *T. castaneum* and recommended *Dracaena arborea* and *Vitex grandifolia* for post harvest control of *T. castaneum*. Ngamo *et al.* (2007) evaluated essential oils of three aromatic plants against four major stored grain insect pests and proved that their natural chemicals caused significant mortality. Essential oils from *Myristica fragrans* and *Illicium verum* showed toxicity against larvae as well as of adults of *T. castaneum* (Shukla, 2008). Tapondjou *et al.* (2005) evaluate essential oils from *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* and *T. confusum* and showed significant mortality on the tested insects. Moreover, plant extracts could be more effective as compared to the individual active compounds, due to natural synergism that delays the development of insecticide resistance to a maximum.

CONCLUSION

Present study was conducted for the assessment of citrus cultivar oils for their possible insecticidal and repellent effects against grub and adult stages of an important pest of stored grains *i.e. T. Castaneum*. Among the citrus cultivars rough lemon showed best results both as insecticidal and for repellency.

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