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Contact Toxicity of Essential Oil of *Citrus reticulata* Fruits Peels Against Stored Grain Pests *Sitophilus oryzae* (Linnaeus) and *Tribolium castaneum* (Herbst)

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Abstract: The effect of volatile compounds of *Citrus reticulata* peel essential oil was studied on the stored grain pest *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). This oil was extracted from the fruit peels using hydrodistillation. Result indicated that essential oil of *C. reticulate* showed toxic effects against these stored grain insect pests. The LC_{50} against the larva of *T. castaneum* was 18.733 µl at 48 h exposure. The LC_{50} against *T. casteneum* and *S. oryzae* adults were 21.638 µl and 19.336 µl at 48 h exposure, respectively.

Key words: *Citrus reticulata* · Essential oil · *Sitophilus oryzae* · Stored grain pests · Toxicity · *Tribolium castaneum*

INTRODUCTION

Cereals and pulses have great nutritional value in developing countries but due attack of insect pest a loss of 20-60 percent have been reported [1, 2]. Control of these insect pest populations around the world primarily depend upon the application of Organophosphorus, pyrethroids and fumigants [3]. However, the potential hazards for mammals from synthetic insecticides, the ecological consequence and the increase of insect resistance to pesticides has led to a search for new classes of insecticides with lower mammalian toxicity and a lower persistent of insecticides in the environment [4]. Therefore, development of bio-insecticides has been focused as a viable pest control strategy in recent years [5, 6]. Many plant are known to have various activities against different stored grain insect pests [7, 8] especially in the form of essential oils that were extracted from aromatic plants have been widely investigated in this connection [9, 10]. The essential oils have the complex mixture of volatile organic compounds that were produced by different plants genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties [11]. The red flour beetle *Tribolium castaneum* Herbst (Coleoptera:Tenebrionidae) and the rice weevil *Sitophilus oryzae* L. (Coleoptera:Curculionide) are the serious pests of different stored grain products [12, 13]. Both the pests are very common, economically important and difficult to control in the field and under storage. For control of insect pests many synthetic chemicals are being used which cause adverse effects on non-target animals in addition to toxicity to the users [14, 15, 16]. The plants volatile essential oils of fruits peels of some citrus species are reported to have insecticidal properties against stored grain insect pests [17-19]. The toxicity of powdered, sun-dried orange and grape fruits peels to *Callosobruchus maculates* has been demonstrated [20].Sweet orange *Citrus sinensis* is a medicinal plant prescribed as traditional medicine to treat diverse illnesses [21]. It has been used as insect repellent, antibacterial and larvicide [22]. The essential oil of *C. sinensis* also has fumigant toxicity against *Aedes aegypti* L. mosquitoes [23]. Considering the importance of plant origin insecticides in the pest management, Present work has been designed to investigate the efficacy of the products of orange peels in the control of the stored grain insect pests, *S. oryzae* and *T. castaneum.*

MATERIALS AND METHODS

Isolation of the Essential Oil: The essential oil was extracted from fruit peels of *Citrus reticulata*. The fruits were collected from the local area of Gorakhpur, (U.P.),

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India during February 2010 to May 2010. The peel was dried in absence of sun light at room temperature 30±5°C and grounded by domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus continuously for five hours to yield the essential oil. The oil was collected in glass containers and kept in appendorff tubes at 5°C until their use.

Rearing of Insects: The red flour beetles *Tribolium castaneum* and the rice weevils *Sitophilus oryzae* were used to determine the toxic property of essential oil. The insects were reared on wheat flour and grain for *T. castaneum* and *S. oryzae*, respectively in the laboratory at 30±2°C, 75±5% r.h. and at photoperiod of 10:14 (L: D) h.

Larval Mortality: Larvicidal property of *C. reticulata* essential oil was tested against newly molted 4th instars *T. castaneum* larvae by contraction. Whatman no. 1 filter papers were cut according to shape of petri dish and treated with solutions of different concentrations, 15, 20, 25 and 30 μl oil in 100 μl acetone using micropipette. The treated filter papers were dried to evaporate the solvent completely. The treated filter papers were placed at the bottom in glass petri dish (height 15 mm × radius 45 mm). Ten larvae of *T. castaneum* taken from the laboratory culture were placed with 1 gram of wheat flour in petri dish. The flour was spread uniformly along the whole surface of the petri dish. All closed petri dishes were kept in dark and six replicates were set for each concentration. After 24 h and 48 h, larval mortality was recorded.

Adult Mortality: The toxic effect of *C. reticulata* essential oil was tested against adults of *T. castaneum* and *S. oryzae* by contraction. The methodology used was the same as that used in determining the toxic effect of larval mortality in *T. castaneum*.

Data Analysis: The LC_{50} was calculated by POLO programme [24]. Correlation and linear regression analysis were conducted to define all dose-response relationships [25]. Analysis of variance was performed to test the equality of regression coefficient [25].

RESULTS

The essential oil of fruits peels of *Citrus reticulata* killed *Tribolium castaneum* larvae and adults of *T. castaneum* and *Sitophilus oryzae* by contact action. The LC_{50} of *C. reticulata* oil was found 22.879 μl and 18.733 μl at 24 and 48 h against larvae of *T. castaneum* (Table 1).The adult toxicity of essential oil against *T. castaneum* 25.795 μl and 21.638 μl at 24 and 48 h whereas 24.471 μl and 19.336 μl at 24 and 48 h exposure against adult of *S. oryzae*, respectively (Table 1).

The regression analysis showed a concentration dependent significant correlation of the oil with larval mortality ($F= 45.727, P<0.01$) at 24 h and ($F=45.271, P<0.01$) at 48 h exposure (Table 2). The concentration dependent significant correlation of the oil with adult mortality of *T. castaneum* ($F=14.051, P<0.01$) and ($F=23.392, P<0.01$) at 24 and 48 h and against *S. oryzae* ($F=27.110, P<0.01$) at 24 h and ($F=56.88, P<0.01$) at 48 h exposure, respectively (Table 2).

Table 1: The toxicity assay of *C. reticulata* essential oil against larva and adult of *T. castaneum* and adult of *S. oryzae*

Insect	Stage	Exposure time (h)	LC50[a] (μl)	LCL-UCL[b] (μl)	g-value[c]	t-ratio[c]	Heterogenity[c]
Tribolium castaneum	Larva	24	22.879	20.24-26.44	0.211	4.265	0.13
		48	18.733	15.47-21.05	0.209	4.29	0.17
	Adult	24	25.795	22.28-34.86	0.331	3.405	0.29
		48	21.638	18.97-24.56	0.205	4.331	0.28
Sitophilus oryzae	Adult	24	24.471	21.87-28.77	0.196	4.426	0.25
		48	19.336	16.25-21.72	0.207	4.304	0.37

[a]LC_{50} represent the median lethal concentration. [b]UCL and LCL represent upper confidence limit and lower confidence limit. [c]g-value,t-ratio and heterogeneity were significant at all probability levels(90%,95%,99%)

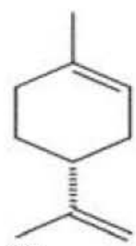
Table 2: Regression parameters of insecticidal effects of *C. reticulata* essential oil against larva and adult of *T. castaneum* and adult of *S. oryzae*

Essential oil	Insect	Stage	Exposure (h)	Intercept	Slope	Regression cofficient	F-value* (df=3.20) P<0.01
Citrus reticulata	*Tribolium castaneum*	Larva	24	-0.81	0.247	0.997	45.727
			48	0.126	0.253	0.981	45.271
		Adult	24	0.815	0.16	0.976	14.051
			48	-0.583	0.249	0.993	23.392
Sitophilus oryzae		Adult	24	-1.321	0.253	0.999	27.110
			48	-0.007	0.250	0.997	56.88

Regression analysis was performed between different concentrations of essential oil and responses of the insect pest. *Significant at 99%probability level.

DISCUSSION

Many essentials oils and their constituents have been studied to posses potential as alternative compound and gaining tremendous importance particularly for the management of stored product ecologically safe and biodegradable [26-29]. Rutaceae is the large family containing 130 genera in seven subfamilies with many important fruits and essential oils product. Lemon essential oil has the highest value of all essential oils and is widely used as flavouring agent in bakery, as fragrance in perfumery and also for pharmaceutical applications [30]. In this study the result showed that insecticidal effect of essential oils from peel of citrus fruits on *Tribolium castaneum* and *Sitophilus oryzae* after 24 and 48 h exposure. This study showed that this essential oil has significant ($P<0.01$) and good toxicity against both stored grain insect pests. Previously for the management of economic loss caused by *T. castaneum* and *S. oryzae* several essential oils of botanical origin have been reported for their insecticidal activies. The essential oil of *Citrus sinensis* showed contact toxicity against *Zabrotes subfasciatus* L. [31]. Essential oil derived from orange peels is known to have toxic, feeding deterrent and poor development effects on lesser grain borer, *Rhyzopertha dominica*(F.), rice weevils, *Sitophilus oryzae* (L.) and red floor beetle, *Tribolum castaneum* (Herbst) [19]. The gas chromatographic analysis of citrus peel oils and components of these oils have been tested against *Callosobruchus maculatus*. Several compounds including the major component of all citrus peel oils, has been found to be bioactive, which have a strong vapour insecticidal activity. A combined study has established that in artificial mixtures several pure components of citrus peel oil potentiate their individual fumigant action in a consistent manner with a preservative model against *C. maculatus* [32]. The peel oil was also reported to have toxicity toward *Culex pipiens* [33]; and cow pea weevils, *Callosobruchus maculates* (F.) [34]. Further more, the peel oil has fumigant action against fleas [35] and house hold insects *Blatella germanica* (L.) and *Musca domestica* (L.) and stored product *S. oryzae* [36]. *Anna senegalensis*, *Hyptis specigera* and *lippie regosa* essential oils were tested against the four major stored product insect pests *Sitophilous zeamais*, *S. oryzae*, *C. maculatus* and *T. castaneum*. *H. specigera* essential oil was the most active towards *S. oryzae*, *T. castenium* was the less sensitive insect against the three essential oils [10]. The essential oils from different parts of plants, fruits of *Schyzygium aromaticum,* leaves of *Aegle marmelos,* seed of *Corriandrum sativum* and peel of *C. reticulata* fruits extracted by a water distillation method showed strongly repellency against *S. oryzae* and *T. castaneum* even at low concentration but its repellency was more marked towards *S. oryzae* [37]. The essential oils extracted from Citrus genus have monocyclic monoterpenoides and its major component is d-limonene (þ-mentha-1,8-dene) and they have insecticidal activity against insect pests [36]. Similarly in the present study the essential oil of *C. reticulata* showed more toxicity at 48 h exposure against larvae and adults of *T. castaneum* and adults of *S. oryzae*.



d-limonene

Varying activity by different essential oils indicated that the pest controlling and repellent factors were not uniformly present in every aromatic plant. Therefore, essential oils from fruit peels of *C. reticulata* may be recommended as cheap, easily available at farmer level, eco-friendly with low mammalian toxicity and good alternative to synthetic insecticides. It could further reduce the application of the synthetic insecticides.

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