Journal of Phytopathology and Pest Management 2(2): 27-33, 2015
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Toxicity of Basil (*Ocimum basilicum* L.) and Rosemary (*Rosmarinus officinali* L.) extracts on *Tribolum confusum* (DuVal) (Coleoptera:Teneberionidae)

Ghada S. Mohamed

Plant Protection Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt

Abstract

This study was conducted to monitor the effect of two-plant extracts viz. leaves of *Ocimum basilicum* L. and *Rosmarinus officinalis* L. on the last larval and adult stages of *Tribolum confusum* (DuVal). Bioassay was made using the ethanol as a solvent for both plants to find out the median lethal concentration (LC_{50}). Results showed that the last larval instar was more highly susceptible to both plant extracts than the adult stages. The LC_{50} of *R. officinali* were 113.15 and 135.18, whereas the LC_{50} of *O. basilicum* extract were 148.38 and 218.78 ppm for the last larval and adult stages after 24 hours exposure respectively. The highest mortality percentages were 78% and 72% for *R. officinali* and 74%, 66% for *O. basilicum* against *T. confusum* last larval instar and adult stages, respectively, after 24 hours exposure at 250 ppm. The application of these botanicals extracts might be promising in protecting the stored grains against coleopteran pests.

Key words: stored-product pest, control, plant extracts, flour beetles, IPM.

* **Corresponding author:** Ghada S. Mohamed,
E-mail: d.ghada1@yahoo.com

Introduction

Stored foods suffer heavily losses during storage due to pests. According to the FAO estimate, 10 to 25% of the world's harvested food is destroyed annually by insects and rodent pests (Anonymous, 1980). Insect pests cause damage to stored grains and processed products by reducing their dry weight and nutritional value (Sinha & Watters, 1985). The confused flour beetle, *Tribolum confusum* (DuVal) is one of the most serious pests of stored cereals and processed cereal products worldwide (Weston & Rattlingourd, 2000). These pests possess a high reproductive rate and therefore effective protection of stored products against their infestation is necessary (Jovanovic et al., 2007; Shukla et al., 2010). Chemical control using synthetic insecticides and fumigants is a common practice used to control pests of stored grains. However, due to development of insect strains resistant to pesticides (Zettler & Cuperus, 1990), toxic residues on stored grain for human consumption, acute and chronic toxicity for workers and adverse effects on the environment; alternative approaches in integrated pest management (IPM) have been considered (Shaaya et al., 1997). In recent years, agrochemical companies have focused on the study of natural products for the development of new pesticides (Addor, 1995). Plant products have for many generations been used on small scale farmers in parts of Africa and Asia to protect stored products from insect infestation (Poswal & Akpa, 1991; Parh et al., 1998). Basil (*Ocimum basilicum* L.) and Rosemary (*Rosmarinus officinali* L.) are considered an aromatic herb, which used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics (Javanmardi et al., 2002). Traditionally, Basil and Rosemary have been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction (Simon et al., 1999). The essential oil of these plants has antifungal, physicochemical and insect repelling activity (Martins et al., 1999). The present study is carried out to determine the toxicity of the leave extracts of *O. basilicum* and *R. officinali* on confused the flour beetle, *T. confusum*.

Materials and methods

Tested Insects: Mass culture for confused flour beetle *T. confusum* was maintained in the laboratory over one year without exposure to insecticides and reared in glass containers (20 cm length x 14 cm width x 8 cm height) containing wheat flour mixed with yeast (10:1,w/w) which were covered by a fine mesh cloth for ventilation. The cultures were maintained in an incubator at 30°C for testing of the last instar larvae and adult insects of *T. confusum* (Jlblou et al., 2006).

Plant materials: Fresh leaves of *O. basilicum* and *R. officinali* were collected from Qena city, Egypt. Leaves of these plants were washed with water, shade dried and ground to a fine powder with an electric blender. About 12.5 gm of the powdered material from each plant was soaked separately, in the dark, in a solution of 12.5 ml water and 50 ml ethanol solvent. After one day the solutions were filtered and stored in the refrigerator prior to use (Al-Lawati et al., 2002).

Bioassay experiment: Different concentrations of the extract (between 50ppm to 500ppm) were prepared using distilled water. Then 5ml solution of each concentration was dropped in Petri dish (9cm diameter) with the help of a pipette and evenly throughout the Petri dish. The Petri dishes were then air dried for a few minutes. Ten confused flour beetles (adult and last larval stage) were put into each Petri dish (five replicate per concentration). Control Petri dishes were treated with the distill water only. After adding (adult and last larval stage), the glass dishes were kept in a laboratory under room temperature. By counting the number of dead (adult and last larval stage) at 24 hrs of exposure, the mortality rate and the median lethal concentrations were obtained LC_{50} by probit regression analysis program and expressed in ppm.

Results and Discussion

The experiment was conducted in order to determine the activity of plant extracts on the adult and last larval instar of *T. confusum*. In all cases, considerable differences in insect mortality were shown with different plant extract, different concentration at the same exposure period 24 hrs. The 24 hrs bioassay is a major tool for evaluating the toxicity of phytotoxins, and a number of researches have been applying this method to assess the toxic effect of different plant extractions against *T. confusum* (Kundu et al., 2007; Hana, 2013). The *T. confusum* larvae that exposed to the plant extracts showed significant behavioral changes. The changes were observed within 30 minutes of exposure. The larvae showed restlessness, loss of equilibrium and finally led to death. These behavioral effects were more pronounced in case of *R. officinali* than *O. basilicum* extracts after exposures. These effects may be due the presence of neurotoxic compounds in the both plants. No such behavioral changes were obtained in control groups.

Table 1: Toxicity of leaf extracts of *O.basilicum* and *R.s officinali* against last instar larvae and adult stage of *T. confusum* after 24 hrs exposures.

Plant extract	Stages of exposure	LC_{50} (ppm)	95% Fiducial limits		Slope	S.E
			Upper	Lower		
R. officinali	Larvae	113.15	138.14	88.79	1.87	0.34
	Adults	135.18	164.84	110.95	2.01	0.35
O. basilicum	Larvae	148.38	176.85	126.14	2.38	0.37
	Adults	218.78	209.20	182.87	2.36	0.41

Lethal concentration of leaf extract was 148.38 ppm (*O. basilicum*) for the last larval stage and it was 218.78 ppm for the adult stage at the 24 hrs of exposure period (Table 1). The leaf extracts of *R. officinali* was more effective than the *O. basilicum* extract. The LC_{50} of *R. officinali* was 113.15 ppm for the last larval stage, whereas it was 135.18 ppm for the adult stage. Zero percent of mortality was noted in the control. The last larval stage and adult mortality rate was higher when exposed to *R. officinali* compared to *O. basilicum* toxicity (Table 2).

Table 2: Mortality percentages of larvae and adults of *T. confusum* at different concentrations of leaf extract of *O. basilicum* and *R. officinali* after 24 hrs exposure.

Plant extract	Stages of exposure	Parameters	Effective concentration in ppm					
			Control	50	100	150	200	250
R. officinali	Larvae	Mortality (%)	0	28	42	58	66	78
	Adults	Mortality (%)	0	22	34	54	64	72
O. basilicum	Larvae	Mortality (%)	0	14	34	48	60	74
	Adults	Mortality (%)	0	8	20	32	44	66

The toxic and repellent effects of phytochemicals on *T. confusum* depend on several factors among which are the chemical composition of the crude plant extracts and insect susceptibility (Casida, 1990). Crude plant extracts contain components that increase their repellency and /or toxicity. The highly toxic and repellent effects of the main constituents of these oil are the cymol, 1,8-cineole, terpineol and a-pinene, which have been demonstrated against *T. confusum* by researchers (Ojimelukwe & Adler, 1999; Tapondjou et al., 2005). Repellency could be due to the blend of oil constituents or to minor compounds present in oils and characterized by a significant biological activity (Cosimi et al., 2009). Various biological activities have been reported for some plant species e.g. *Mentha*, antibacterial (Oyedeji & Afolayan, 2006), antifungal (Bouchra et al., 2003), insecticidal properties (Akrami, 2008) and repellency effect against storage pest (Mahmoodvand, 2012; Saeidi & Moharramipour, 2013). Many researchers pointed that some of plant essential oils showed strong repellency effects against storage pests (Liu & Ho, 1999; Akrami, 2008; Caballero-Gallardo et al., 2012; Saeidi & Moharramipour, 2013). Based on the result from the bioassay test, the tested crude plant extracts showed a high toxicity when it was applied against insects. The insecticidal activity depends on the crude plant extracts concentration and insect stage. A concentration of 113.15 ppm of *R. officinali* extract was necessary to cause 50% mortality for the last larval stage, while a concentration of 135.18 ppm for the adult stage. The same result obtain in *O. basilicum* extract when concentration of 148.38 ppm of *O. basilicum* extract was necessary to cause 50% mortality for the last larval stage; a concentration of 218.78 ppm for the adult stage (Table 1). Moreover, slopes estimated that any increase in crude plant extracts concentration, inflicted the highest mortality on last larval stage when compared with the adult. The experiment has shown that last larval stage were more susceptible than the adult (Table 1, 2). These results are in agreement with those reported earlier and indicated that the insecticidal activity of crude plant extracts varies depending on the stage of the insect (Papachristos & Stamopoulos, 2002; Isikber et al., 2006). Huang et al. (2000) which previous researcher tested the major constituents of the essential oil of *Allium sativum* (L.) against larvae of the *Tribolium castaneum.* They reported that younger larvae were more susceptible than older larvae to the toxicity of these compounds. The researchers determined the LC_{50} and observed the behavioral changes and mortality in the insect stages. Similar observations were noticed in the present study and support the potential application of these herbs in confused flour beetle control measures. The effect of *R. officinali* and *O. basilicum* extracts have already been reported against confused flour beetle (Mona & Yasser, 2009; Saeidi & Moharramipour, 2013). As the leaf extract of *R. officinali* and *O. basilicum* is a highly toxic even at low doses, these plants may eventually prove to be useful as a larvicide (Mahdieh & Saeid, 2013). Further analysis is required to isolate the active constituents and to determine the optimum dosages which serve as a larvicidal and adult emergence inhibition against *T. confusum*. In conclusion, the product of these plants can be well utilized for preparing biocides or phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants would be an eco-friendly and a suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and readily available in many areas of the world.

References

Addor RW, 1995. Insecticides. Agrochemicals from Natural Products. In: Godfrey CRA, ed., Marcel Dekker Inc., New York, USA, pp: 1-63.

Akrami H, 2008. Insecticidal effect of essential oils from *Thymus kotschyanus* and *Mentha longifolia* on some stored product Insects. MSc Thesis of Entomology, Islamic Azad University, Science and Reserch Branch, Iran, 97 pp.

Al-Lawati HT, Azam KM, Deadman ML, 2002. Potential of Omani Flora as Source of Natural Products for Control of Pulse Beetle, *Callosobruchus chinensis*. Agricultural Sciences **7**: 59-63.

Anonymous, 1980. Introduction to Detia. Fumigation Detia export GmH, 3.

Bouchra C, Achouri M, Idrissi-Hassani LM, Hmamouchi M, 2003. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea.* Journal of Ethnopharmacology **89**: 165-169.

Caballero-Gallardo K, Olivero-Verbel J, Stashenko EE, 2012. Repellency and toxicity of essential oils from *Cymbopogon martinii*, *Cymbopogon flexuosus* and *Lippi aoriganoides* cultivated in Colombia against *Tribolium castaneum*. Journal of Stored Products **50**: 62-65.

Casida JH, 1990. Pesticide mode of action, evidence for implications of a finite number of biochemical targets. In: Casida, JE ed., Pesticides and Alternatives: Innovative Chemical and Biological Approaches to Pest Control, Elsevier, Amsterdam, pp. 11–22.

Cosimi S, Rossi E, Cioni PL, Canale A, 2009. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored product pests: Evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). Journal of Stored Products **30**: 1–8.

Hana HM, 2013. Repellency of ethanolic extract of some indigenous plant against *Tribolium confusum* (DuVal) (Coleoptera; Tenebrionidae). Journal of Agriculture and Veterinary Science **6**: 27-31.

Huang Y, Chen XS, Ho HS, 2000. Bioactivities of methyl and allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of storedproduct pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology **93**: 537−543.

Isikber AA, Alma MH, Kanat M, Karci A, 2006. Fumigant toxicity of essential oils from *Laurus nobilis* and *Rosmarinus officinalis* against all the stages of *Tribolium confusum*. Phytoparasitica **34**: 167−177.

Javanmardi J, Khaleghi A, Kashi A, Bais HP, Vivanco JM, 2002. Chemical characterization of Basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. Journal of Agriculture and Food Chemistry **50**: 5878-5883.

JIblou R, Ennabili A, Sayah F, 2006. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbest) (Coleoptera; Tenebrionidae). African Journal of Biotechnology **10**: 936-940.

Jovanovic Z, Kosti M, Popovic Z, 2007. Grainprotective properties of herbal extract against the bean weevil *Acanthoscelides obtectus* Say. Industrial Crops and Products **2:** 100-104.

Kundu BR, Begum R, Ara MM, Sarker ZI, 2007. Effect of Bishkatali, *Polygonum hydropiper* L. plant extracts against the red flour beetle, *Tribolium castaneum* (Herbst). Journal Zoological Rajshahi University **26:** 93-97.

Liu ZI, Ho SH, 1999. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). Journal of Stored Products **35**: 317-328.

Mahdieh S, Saeid M, 2013. Insecticidal and repellent activities of *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* essential oils on *Tribolium confusum*. Journal of Crop Protection **1:** 23-31.

Mahmoodvand S, 2012. Effects of essential oil and powder four plant species on *Tribolium castaneum* (Herbst) and *Tribolium confusum* (DuVal) (Coleoptera: Tenebrionidae). MSc Thesis, Islamic Azas University, Arak Branch.

Martins AP, Salgueiro LR, Vila R, Tomi F, Canigueral S, Casanova J, Proença DA, Cunha A, Adzet T, 1999. Composition of the essential oils of *Ocimum canum*, *O. gratissimum* and *O. minimum*. Planta Medica **65:** 187–189.

Mona FA, Yasser AE, 2009. Toxicity and biochemical efficacy of six essential oils against *Tribolium confusum* (du val) (Coleoptera: Tenebrionidae). Egyptian Academic Journal of Biological Sciences **2:** 1 – 11.

Ojimelukwe PC, Adler C, 1999. Potential of Zimtaldehyde, 4-allyl-anisol, linalool, terpineol and other phytochemicals for the control of confused flour beetle (*Tribolium confusum* J.D.V.). Journal of Pesticide Science **72**: 81–86.

Oyedeji OA, Afolayan AJ, 2006. Chemical composition and antibacterial activity of the essential oil isolated from South African *Mentha longifolia* (L.) subsp. *capensis* (Thunb.) Briq. Journal of Essential Oil Research **18:**57-59.

Papachristos DP, Stamopoulos DC, 2002. Toxicity of vapours of three essential oils to the immature stages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Products Research **38:** 365−373.

Parh IA, Forbuzo BC, Matheney EL, Ayafor JF, 1998. Plants used for the control of insect pests on stored grains in parts of North West Highland Savana zone of Cameroon. Science Agronomiques et Developement **1:** 54-60.

Poswal MAT, Akpa AD, 1991. Current trends in the use of traditional and organic methods for the control of crop pests and diseases in Nigeria. Tropical Pest Management **37:** 329-333.

Saeidi M, Moharramipour S, 2013. Insecticidal and repellent activities of *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* essential oils on *Tribolium confusum*. Journal of Crop Protection **2:** 23-31.

Shaaya E, Kostjukvski M, Eilberg J, Sukprakarn C, 1997. Plant oils as fumigants and contact insecticides for the control of stored product insect. Journal of. Stored Products Research **33**: 7-15.

Shukla GS, Upadhyay VB, Mathur R, Prasad SG, 2010. Economic zoology, biostatistics and animal behaviour. Rustogi Publication, India, pp: 97.

Simon JE, Morales MR, Phippen WB, Vieira RF, Hao Z, 1999. A source aroma compounds, a popular culinary and ornamental herb. In: Janick J, ed. Perspectives on new crops and new uses: 499-505.

Sinha RN, Watters FL, 1985. Insect pests of flour mills, grain elevators, and feed mills and their control. Agriculture Canada, Publication 1776, Ottawa, ON, Canada.

Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C, 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* DuVal. Journal of Stored Products Research **41:** 91–102.

Weston PA, Rattlingourd PL, 2000. Progeny production by *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensi* (Coleopter:Silvanidae) on maize previously infested by *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Journal of Economic Entomology **93:** 533-536**.**

Zettler JL, Cuperus GW, 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology **83**: 1677-1681.