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Insecticidal Activity of Essential Oil of *Thymu Vulgaris* on the *Callosobruchus Maculatus*

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Abstract: *Plant derived insecticides as compared to synthetic ones are safer for the environment, generally cost effective, easy to handle and used by small industries and farmers. an experiment was conducted on Hamedan Agricultural Research Center, , as factorial based on completely randomized blocks in 3 replications in 2009 during. Experimental treatments included 6 levels with concentration (0, 0.25,0. 50,0.75,10,20,30) mg/cm^2 as the time at 3 levels (8,24,48) hours as the second factor. 1 ml of each solution were applied on filter papers (Whatman No. 1). Then each dried paper was placed at the bottom of a Petri dish (5.5 cm × 1.2 cm) and 10 adults each of C. maculatus was placed in each Petri dish and covered with a lid. Controls received only water alone. Each set of treatment was repeated 3 times and number of dead insects in each petri-dish was counted at an interval of 8, 24, 48 hour respectively. Percentage mortality was calculated formula. The findings of contact toxicity unveiled that the mode of action of essential oil and its components against adults of C. maculatus was dosage and exposure time dependent. It has been noticed that the Thymu vulgaris essential oil showed 56% mortality adult C. maculatus at a dose of 0.25 mg/cm^2 respectively. Further increase the concentration of oil to 30 mg/cm^2, yielded higher mortality of 98% against adult C. maculatus. No mortality was obtained in the control within the same time period.*

Keywords: *Callosobruchus maculatus, Thymu vulgaris, Essential Oil*

1. Introduction

Stored insect pests are a major problem throughout the world as they significantly reduce the quantity and quality of grain. The post-harvest grain losses due to insect pests and other bio-agents ranged from 10 to 40% (Raja et al., 2001; Papachristos and Stamopoulos, 2002). It is evident from literature that the cowpea weevil, Callosobruchus maculatus (F.) and the rice weevil, Sitophilus oryzae (L.) are considered as the most widespread and destructive primary insect pests of stored legumes and cereals (Park et al., 2003). The main method of grain protection and to avoid or control insect infestations is the use of chemical agents since, it is the simplest and most cost effective means of dealing with stored product pests. But the excessive use of traditional chemical insecticides leads to a number of serious problems, like persistence in the atmosphere, resistance to chemical insecticides, pests resurgence, exclusion of economically beneficial insects, toxicity to humans and environment and higher cost of crop production. So, there is an urgent need to develop safe alternatives to conventional fumigants and insecticides to control the insect infestation in stored grain products. Continued screening for such systems of grain protection that target the pest species more accurately is required (Cox, 2004). Plant derived insecticides as compared to synthetic ones are safer for the environment, generally cost effective, easy to handle and used by small industries and farmers. Botanical pesticides are often active against a number of species, are often biodegradable, nontoxic and appropriate for use in integrated pest management (Kim et al., 2003). In this context, plant extracts including essential oils can play an important role in protecting stored products against insect infestations. Essential oils derived from plants are volatile in nature and their constituents have revealed adequate activity as botanical pesticides (Singh and Upadhyah, 1993). Spices are considered as rich sources of essential oils and are known to be effective against various insect pests including stored product insect pests (Jacobson, 1989). Clove oil was found toxic to S. oryzae and castor oil to C. maculatus and C. phaseoli in stored conditions. Fumigant toxicity of essential oils from several spices like anise

(Pimpinella anisum L.) and peppermint (Mentha piperita L.) was found against four major stored product pests including, Ryzopertha dominica (F.), Tribolium castaneum (Herbst), S. oryzae and Orzyaephilus surinamensis (L.) (Shaaya et al., 1991). In this work, we have examined the activity of essential oil from Thymu vulgaris on the Callosobruchus.

2. Materials and Methods

2.1. Stock Culture

The *Callosobruchus maculatus* (Fabricus) was used in the present investigation. A small population of beetles was reared and bred under laboratory conditions on the seeds of cowpea (Vigna unguiculata) inside a growth chamber at 30+ 20C, 12:12 L: D and with 70% RH.

2.2. Plant Material

Samples of *Thymu vulgaris* were collected during in May, 2009. The dried aerial parts were submitted to Hydro distillation for 3 h using Clevenger type apparatus, according to the European Pharmacopoeia (European Pharmacopoeia, 1996). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used.

2.3. Mass Spectrometry Analysis

The oil was analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph. The MS operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass unit were monitored from 30 to 450 m/z . Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY275.L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the lite rapture (Adams, 1995).

2.4. Contact Toxicity

Insecticidal activities of essential oil of *Thymu vulgaris* adults of *C. maculatus* has been determined by direct contact application as cited in literature (Kim et al. 2003; Usha Rani 2010). *Thymu vulgaris* oil were prepared at concentrations of (0.25,0.50,0.75,10,20,30 mg/cm^2) respectively. 1 ml of each solution was applied on filter papers (Whatman No. 1). Then each dried paper was placed at the bottom of a Petri dish (5.5 cm × 1.2 cm) and 10 adults each of *C. maculatus* was placed in each Petri dish and covered with a lid. Controls received only water alone. Each set of treatment was repeated 3 times and number of dead insects in each petri-dish was counted at an interval of 8, 24, 48 hour respectively. Percentage mortality was calculated formula.

2.5. Statistics Analysis

Statistical analysis was carried out using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) and Comparing averages were carried out by one-way ANOVA using Duncan test.

3. Results

3.1. Chemical Composition of the Essential Oil

Essential oil yield was 1.0%. Freshly isolated essential oil was a yellow liquid with intensive, narcotic odour. The components of essential oil were separated into five classes, which were monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others . Based on GC and GC-MS analysis of the essential oil of Thymu *vulgaris* 40 components were identified. Among those, isospathulenol (21.68%), α-Humulene epoxide II (21.04%), Caryophellene oxide (20.45%), Spathulenol (20.42%), Viridiflorene (18.34%) were the major oil components (figure 1).

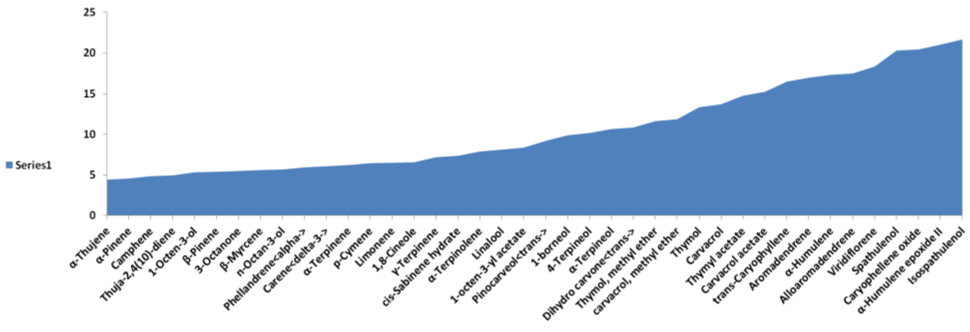

Figure1. *Chemical composition of Thymus vulgaris L*

3.2. Contact Activity with Treated Filter Paper

The results of comparing the means with Duncan's test indicated that there was a significant difference between different levels of essential oil of *Thymu vulgaris* with control (Table 1). The results of the analysis of variance showed that treatment of essential oil of *Thymu vulgaris* had significant effect on death rate of *Callosobruchus maculatus* at probability level of 1%. Findings of essential oil the experiment indicated that the rate of losses of *Callosobruchus maculatus* had the lowest amount in all experiments in control treatment (no use of *Thymu vulgaris* essential oil). The findings of contact toxicity unveiled that the mode of action of essential oil and its components against adults of *Callosobruchus maculatus* was dosage and exposure time dependent. Concentrations 30 mg/cm^2 the highest percentage of deaths. The highest effects were observed in 48 hours after adding the extract. The contact activity method has been widely used to investigate the toxicity of insecticides. The findings of contact toxicity unveiled that the mode of action of essential oil and its components against adults of *C. maculatus* was dosage and exposure time dependent. It has been noticed that the Thymu *vulgaris* essential oil showed 56% mortality adult C. maculatus at a dose of 0.25 mg/cm^2 respectively. Further increase the concentration of oil to 30 mg/cm^2, yielded higher mortality of 98% against adult *C. maculatus*. No mortality was obtained in the control within the same time period. Results in figure 2 showed strong toxicity the insect species than the Thymu vulgaris oil. In a test with C. maculatus adults, *Thymu vulgaris* at a dose of 30 mg/cm2, caused 98 and 100% mortality at the time interval of 24 and 48 hour treatment respectively.

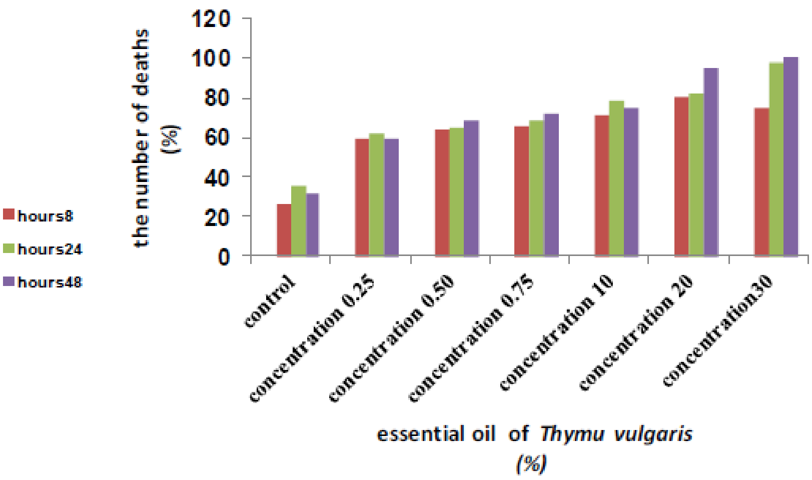

Figure2. *Compares the average the number of Insecticidal Activity of essential Oil of Thymu vulgaris on the Callosobruchus maculates*

Table1. *Analyze variance of data squares of Insecticidal Activity of essential Oil of Thymu vulgaris on the Callosobruchus maculatus*

S.O.V	Df	M s (Number of losses)
Replication	2	12.81
Essential oil (A)	6	53.54**
hours (B)	2	15.46**
A × B	12	305.45**
Error	40	10.12
%CV		13.02

4. Discussion

Essential oil are natural products that contain natural flavors and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odors (Mahdi et al., 2011). Essential oil components and quality vary with geographical distribution, harvesting time, growing conditions and method of extraction (Yang et al., 2005). Among the essential oil components, the monoterpenoids have drawn the greatest attention for insecticidal activity against stored-product insects. Many essential oils isolated from various plant species belonging to different genera, contain relatively high amount of monoterpenes (Ogendo et al., 2008). Monoterpenes are volatile and responsible for the characteristic odours of many plants. Their volatility which made them easy to discover in fragrant plant material and at the same time readily obtainable by simple distillation of plant parts, lent to them the essential oil (Ibrahim, 2001). These are easily degradable in soil and water (Misra and Pavlostathis, 1997). Previous studies have shown that the toxicity of essential oils obtained from aromatic plants against storage pests is related to the oil's main components (Isman et al., 2001; Lee et al., 2003) such as 1.8 Cineole, Carvacrol, Thymol, Eugenol, Terpinene, Limonene, α-Pinene, among others. The essential oil of a plant may contain hundreds of different constituents but certain components will be present in larger quantities. 1,8-cineole was predominant in the essential oils of Achillea millefolium (22%), Artemisia aucheri (22.8%), Eucalyptus camaldulensis (69.46%), Eucalyptus globulus (31.42%), Lavandula stoechas (48.5%), Laurus nobilis (4.02) and Perovskia atriplicifolia (20.74). In recent years, several studies were reported on the toxicity of some essential oil constituents against various insect species. Obeng-Ofori et al. (1997) found 1,8-cineole to be highly repellent and toxic to Sitophilus granarius L., S. zeamais, Tribolium confusum du Val and Prostephanus truncatus (Horn). Antifeedant activity of 1,8-Cineole has been demonstrated against T. castaneum (Tripathi et al., 2001). Application of 1, 8-Cineole reduced oviposition rate by 30-50% at concentration of 1.0%, as compared to untreated controls (Koschier and Sedy, 2001). Lee et al. (2002) reported that 1,8-cineole was the most toxic fumigant constituent against the adults of Tribolium castaneum Herbst. In pest management strategies, aromatic plants with long lasting insecticidal efficiency should be considered. These considerations must take into account the pest species or the type of stored products. Large quantities of plant material would need to be processed to gain enough essential oil for commercial-scale tests. However, certain compounds in the oils exhibit much stronger activity than others. Plant varieties should be sought that produce these compounds in larger quantities, or synthetic production methods should be explored as an option to gain enough material for full-scale use. From the above discussion, it is clear that essential oils possess a wide spectrum of biological activity against insects and provides a simple and environment friendly (non-polluting and lesser or no toxicological concerns) alternative pest control. Since essential oils have strong toxicity in the vapour form against a wide range of insects, they could be commercially exploited as a fumigant for stored products and also impregnated into packaging thus preventing the insect infestation. However, the effects on other non-target microorganisms including pollinators, honeybees and natural predators/enemies have not been yet evaluated. If the problem of cost-effective commercial production can be solved, some of the essential oils and their compounds could be find a place in IPM strategies, especially where the emphasis is on environmental and food safety and on replacing the more dangerous and toxic fumigants and insecticides.

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