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EFFECT OF FOUR PLANT EXTRACTS AGAINST *TROGODERMA GRANARIUM* AND *TRIBOLIUM CASTANEUM*

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Abstract

The storage of food products and grains against creepy-crawly creatures that damage the crops is a serious problem throughout the World. The study was conducted to evaluate the effect of four plants extract Peppermint (*Mentha piperita*), Thyme *(Thymus vulgaris*), Rosemary (*Rosmarinus officinalis*) and Lemon blam (*Melissa officinalis)* against two pests *Trogoderma grananium* and *Tribolium castaneum* to check the percent mortality rate of larvae and adults. For the preparation of extract fresh leaves of concern plants were collected and dried. The dried leaves were grinded and each plant samples (5grams) were soaked in 100 ml of ethanol. The liquid extracts were filtered and poured for experimental purpose. Three replications were made for each treatment. Statistical analysis was done by RCB method and package used for calculation was M-statC. Results revealed that all the plants extract had lethal effect against adults and larvae of both stored pests as compared to control treatment. *Rosmarinus officinalis* extract was the most efficient against the adults of both stored grain pests, causing 58.67% mortality in *Tribolium castaneum* and 80.00% mortality in *Trogoderma grananium*. Similarly, *Rosmarinus officinalis* also showed maximum mortality against *Tribolium castaneum* larvae (58.67%) and *Trogoderma grananium* larvae (65%). It is concluded from the above results that *Rosmarinus officinalis* showed the highest mortality rate against larvae and adult of both stored grain pests. Insecticidal effects of plants extract have been considered to be most effective and accessible to control several insect pests. This biological method for controlling stored grain pest is an efficient technique as compared to chemical method because of their high toxicity that affects the quality of crop and very costly which is not affordable.

Key words: Mortality, Pest, Stored grains, Plant extract*, Tribolium castaneum, Trogoderma grananium.*

Introduction

The storage of food products and grains against creepy-crawly creatures that damage the crops is a serious problem (Haq *et al.,* 2005). It is being calculated that approximately 11 to 26% of the globe harvested food is shattered per year by insects and pests. According to the recent estimation about 8% of total grain production of the world is get affected by insects and pest infestations. Due to this problem there is an imperative need to build up friendly environmental techniques which must have ability to substitute the highly toxic chemicals. Damage of many stored food products and grain is a very serious problem throughout the globe especially in developing countries (Hasan *et al.,* 2006). Many of the stored grain products get affected from insects; they not only damage grains in stores but also during shipping and transportation of stored grains. It has been estimated that about 10- 40% stored grains in granaries houses and products get damaged due to these pests. Moreover, these insects also reduce the quality of grains (Shaaya *et al.,* 2009). Many pests affect the embryos of stored grain that reduce the protein content of particular grain as well as it also lowers the germination percentage of seeds (Upadhyay & Ahmad, 2011). Among many stored grain pest, *Trogoderma granarium* commonly named as khapra beetle, (a member of Order Coleoptera, Family Dermestidae) is thought to be one of the most a severe primary pest of foodstuffs which may include various leguminous crops. It is originated from India and then spread to other continents like Africa, Europe, South America and East Asia (Hasan *et al.,* 2006). Khapra beetle, *T. granarium* affect both quality and quantity of wheat during storage. *Trogoderma granarium* is a major threat to stored wheat, which is considered as one of the 100 most invasive pests in the world. It is reported that damages caused by *T. granarium* range from 0.3 to 2.6% over a period of 2-11 months of storage (Irshad *et al*., 1988). This beetle sustains its presence in very small numbers and is capable to survive during long period of time in a dormant state (Dwivedi & Shekhawat, 2004). The rate of development and survival of different stage of khapra beetle vary considerably and it depends upon light, moisture, season, temperature and the species of host. Due to the result of high humidity *Trogoderma granarium* have 1-9 or more generation within one year (Ramzan & Chahal, 1986). Under favorable condition and temperature the egg, pupa and adult take about a week to develop, but larvae may take a month to develop into adult. Important stored grain pest *Tribolium castaneum* also known as red flour is belongs to Family *Tenebrionidae.* It is a worldwide pest of stored products (Weston & Rattlingourd, 2000). The *Tribolium castaneum* is of Indo-Australian origin and found in temperate areas, but in fact they survive in winter in only protected places, especially where there is a central heating system (Tripathi *et al.,* 2001). *Tribolium castaneum* attacks stored grain & other food products including flour, cereals, pasta, biscuits, beans and nuts causing loss and damage. It may cause an allergic response, but is not known to spread disease or cause damage to structures and furniture (Belmain *et al.,* 2001). A long history of using plant extracts to control insect pests and the compounds responsible for activity (Isman, 2006). Many plants extract containing essential oils and several bioactive chemicals which are toxic to stored-product pests

including *Tribolium castaneum* and *Trogoderma granarium.* The objective of present study was to check the efficacy of four different plant extracts Peppermint (*Mentha piperita*), Thyme (*Thymus Vulgaris*), Rosemary (*Rosmarinus officinalis*) and Lemon blam (*Melissa officinalis)* against two pests *Trogoderma grananium* and *Tribolium castaneum.* Botanical plants extracts are safe for the environment, easily used by farmers and affordable (Belmain *et al*., 2001). These botanical insecticides are more efficient against a restricted number of pests, easily biodegradable to certain harmless products and could lead to the development of diverse safe pest control agents (Kim *et al*., 2003).

Material and Methods

This research was conducted at research laboratory of Entomology, Sardar Bahadur Khan Women's University Quetta during March-December 2015.

Collection of plants: Fresh leaves of four plants, Peppermint (*Mentha piperita*), Thyme (*Thymus vulgaris*), Rosemary (*Rosmarinus officinalis*) and Lemon blam (*Melissa officinalis)* were collected in March, 2015 from Arid Zone Research Centre, Quetta.

Preparation of plant extract: For the preparation of plants extract fresh leaves of all concern plants were collected and air dried at room temperature. The dried leaves of concern plants were grinded and each plant sample (5g) was soaked in 100 ml of ethanol. Flasks were kept at room temperature for 10 days and covered with aluminum foil in order to prevent it from evaporation. The mixtures were shaken vigorously at 12-hour intervals to ensure proper soaking of the plant products. After 10 days each extract was finally evaporated to dryness. The residue was weighted and re-dissolved in the 100 ml ethanol again for 10 days. In the last session liquid extracts were filtered by using a fine cotton cloth and poured into spray bottles. These spray bottles were kept in the refrigerator for experimental purpose (Panezai *et al.,* 2015).

Bioassays: Glass jars were used (capacity 100 ml) for testing the effect of plant extracts against the mortality of *Trogoderma granarium* and *Tribolium castaneum*. All jars were sprayed with plant extracts except control group (not treated) before filling with wheat grains. All jars were filled with wheat (10g) and last larval instars of each insect were collected from infested wheat and released in the jar. The mouth of each jar was covered with muslin cloth to avoid escaping of larvae of concern insects. In total there were five treatments including control one. There were three replications for each treatment. Same procedure was applied for newly emerged adults. The mortality data was recorded at different timings after exposure at an interval of 24 hours (Omar *et al.,* 2012).

Statistical analysis

The data for percent mortality was subjected to statistical analysis using RCB. The package, used for the calculations, was M-StatC. LSD test (least significant test) was used for comparing means (Steels & Torrie, 1960) at the 5% significance level.

Results

The effect of different treatments has been carried out to test out the percent mortality on both the insects. Mean percent mortality of *Trogoderma granarium* and *Tribolium castaneum* was evaluated against different extract on different timings.

Percent mortality of *Trogoderma granarium* and *Tribolium castaneum* larvae: The data shows that mean percent mortality of *Trogoderma granarium* larvae against different experimental extract had significant effect on the mortality of *Trogoderma granarium* larvae. Maximum mean percent mortality was found in *Rosmarinus officinalis* extract which was 65.00%. *Rosmarinus officinalis* showed significant difference with *Thymus Vulgaris* and *Melissa officinalis*, whereas, non-significant difference with *Mentha piperita*. *Mentha piperita* extract showed second highest mortality with the mean percent mortality of 50%, which was non-significantly different from *Melissa officinalis*, *Thymus Vulgaris* and *Rosmarinus officinalis*. The average mortality of 36.67% was found in *Melissa officinalis*, which was significantly different from *Rosmarinus officinalis*, but had non-significant difference with *Thymus Vulgaris* and *Mentha piperita*. Minimum percent mortality of 33.33% was noticed in *Thymus Vulgaris* extract which showed non-significant difference with *Melissa officinalis* and significant difference with *Rosmarinus officinalis*. In control treatment no mortality was recorded which show significant differences with all plant extracts (Fig. 1; Table 1).

The mean percent mortality of *Tribolium castaneum* larvae against different treatments had significant effect on the mortality of *Tribolium castaneum* larvae. Highest mean percent mortality was shown by *Rosemarinus officinalis* which was 58.67%. It showed significant difference with *Melissa officinalis* and *Mentha piperita* and non-significant difference with *Thymus vulgaris*. Second highest mean percent mortality was shown by *Thymus vulgaris* which was 42.67%. It showed non-significant difference with *Melissa officinalis*, *Mentha piperita and Rosemarinus officinalis*. The average mortality was found as 36% in the *Mentha piperita*. It is significantly different from *Rosemarinus officinalis* and non-significantly differs from *Melissa officinalis*, and *Thyme vulgaris*. Minimum percent mortality of 26.67% was shown by *Melissa officinalis* extract which is significantly different from *Rosemarinus officinalis* and non-significantly difference from *Mentha piperita* and *Thyme vulgaris*. Control treatment shows non-significant difference with *Melissa officinalis* while show significant differences with all other plant extracts.

Percent-mortality-of-*Trogoderma-granarium* and *Tribolium-castaneum*-adult: The data shows that mean percent mortality of *Trogoderma granarium* adult against different experimental extract had significant effect on the mortality of *Trogoderma granarium* adult. Maximum mean

percent mortality of 80% was shown by *Rosmarinus officinalis* extract. *Rosmarinus officinalis* had significant difference with *Thymus Vulgaris* and *Melissa officinalis*, whereas it had non-significant difference with *Mentha piperita*. The extract of *Mentha piperita* showed the mean percent mortality of 71.67% which was significantly different from *Melissa officinalis*, *Thymus Vulgaris*, and showed non-significant difference with *Rosmarinus officinalis*. The average mortality was found in *Thymus Vulgaris*, which was 50% and significantly different from *Rosmarinus officinalis* and *Mentha piperita*, but had non-significant difference with *Melissa officinalis*. Minimum mean percent mortality of 50.00% was noticed in *Melissa officinalis* extract which showed non-significant difference with *Thymus vulgaris* and significant difference with *Rosmarinus officinalis* and *Mentha piperita* (Fig. 2; Table 2).

The mean percent mortality of *Tribolium castaneum*, adults against different treatments revealed that all extracts showed a significant effect on the mortality of *Tribolium castaneum*. The highest mean mortality was shown by *Rosmarinus officinalis* which was 58.67% and had significant difference with *Melissa officinalis* while, non-significant difference with *Mentha piperita* and *Thyme vulgaris*. *Melissa officinalis* extract exhibited 42.67% mortality and had non-significant differences with *Mentha piperita* and *Thymus vulgaris*. The average mortality was found as 40% in *Mentha piperita* which showed non-significant differences with all other plant extracts. The minimum mortality of 24% was observed in *Thymus vulgaris* which also had non-significant differences with other plants. In control treatment the mean percent mortality was 9.33%.

Percent-mortality-*Trogoderma granarium*-and-*Tribolium-castaneum*-on-different-hours: The mean percent mortality of *Trogoderma granarium* larvae observed on different hours showed that the exposure time had significant effect on the mortality of *Trogoderma granarium* adult. Highest mortality was found after the exposure time of 96 hours with the mean mortality of 78.67% in larvae and 75.54% in adults. The exposure time of 72 hours has second mean mortality of 56.78% in adult and 53.33% in larvae. The average mean mortality of 49.56% in adult and 46.67% in larvae was noticed in 46 hours. Minimum mean mortality of 32.00% is noticed after 24 hours. The result revealed that mortality was increased with increasing time (Fig. 3; Table 3).

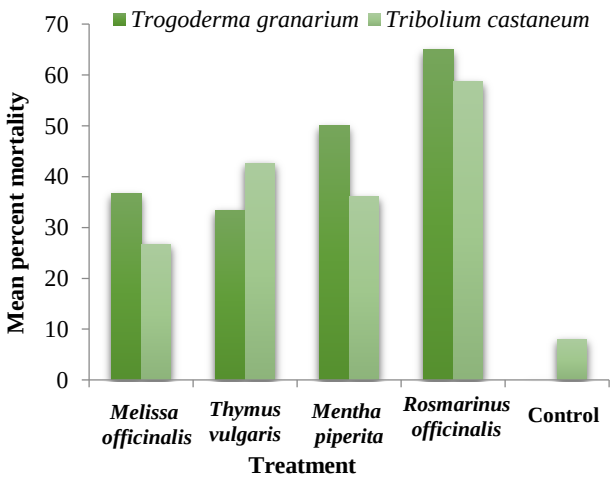

Fig. 1. Effect of treatments on morality rate of *Trogoderma-granarium* and *Triboliumcastaneum* larvae.

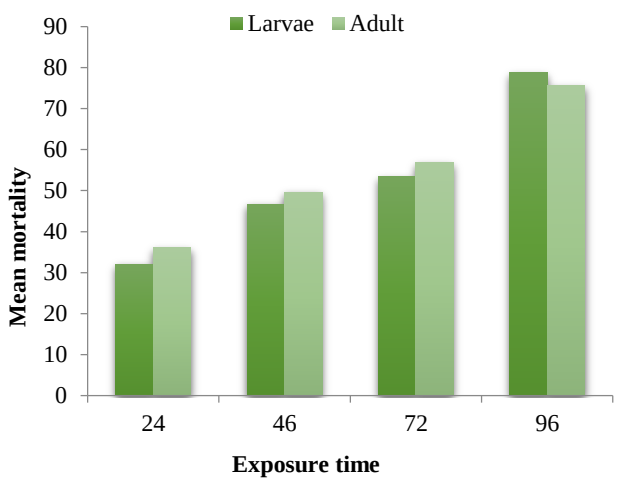

Fig. 3. Effect of botanical extracts on mortality rate of *Trogoderma granarium* (larvae and adult) at different hours.

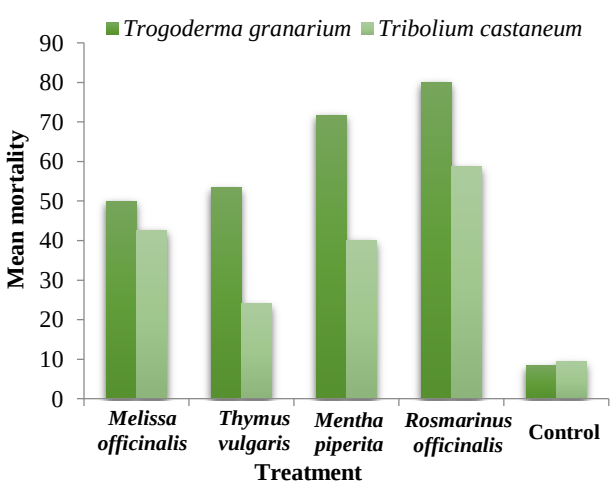

Fig. 2. Effect of treatments on morality rate of *Trogoderma-granarium* and *Tribolium castaneum* adult.

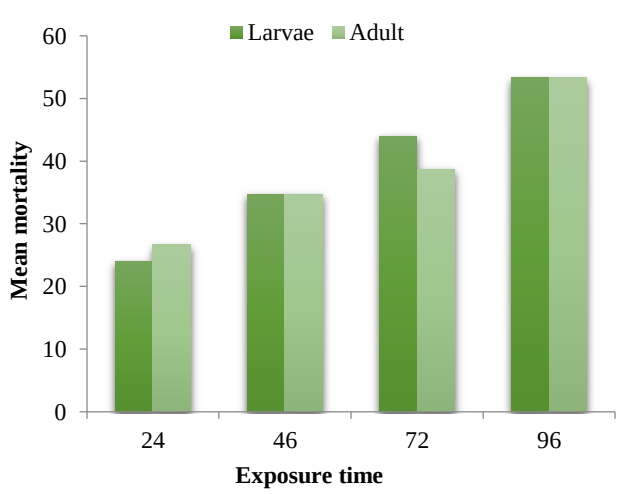

Fig. 4. Effect of botanical extracts on mortality rate of *Tribolium castaneum*a (larvae and adult) at different hours.

Table1. Mean percent mortality of *Trogoderma granarium* and *Tribolium castaneum* larvae against treatments.

Treatment	Mean mortality of *Trogoderma granarium* larvae	Mean mortality of *Tribolium castaneum* larvae
Lemon blam (*Melissa officinalis)*	36.67 B	26.67 BC
Thyme (*Thymus vulgaris*)	33.33 B	42.67 AB
Peppermint (*Mentha piperita*)	50.00 AB	36.00 B
Rosemary (*Rosmarinus officinalis*)	65.00 A	58.67 A
Control	0.00 C	8.00 C

Trogoderma-granarium-LSD-for-treatment-(T)-=-19.55
Tribolium castaneum LSD for treatment (T) = 20.96

Table 2. Mean percent mortality of *Trogoderma granarium* and *Tribolium castaneum* adult against different treatments.

Treatment	Mean mortality of *Trogoderma granarium* adult	Mean mortality of *Tribolium castaneum* adult
Lemon blam (*Melissa officinalis)*	50.00 B	42.67 BC
Thyme (*Thymus Vulgaris*)	53.33 B	24.00 AB
Peppermint (*Mentha piperita*)	71.67 A	40.00 AB
Rosemary (*Rosmarinus officinalis*)	80.00 A	58.67 A
Control	8.33 C	9.33 C

Trogoderma-granarium-LSD-for-treatment-(T)-=-12.31
Tribolium castaneum LSD for treatment (T) = 19.13

Table 3. Mortality of *Trogoderma granarium* against different treatments at different hours.

Exposure time	Mean mortality of larvae	Mean mortality of adult
24 hours	32.00 C	36.00 C
46 hours	46.67 B	49.56 B
72 hours	53.33 B	56.78 B
96 hours	78.67 A	75.54 A

LSD-value-of-larvae-for-hours-(H)-=-11.01
LSD value of adults for hours (H)-= 9.81

Table 4. Mortality of *Tribolium castaneum* against different treatments at different hour.

Exposure time	Mean mortality of larvae	Mean mortality of adults
24 hours	24.00	26.67
46 hours	34.67	34.67
72 hours	44.00	38.67
96 hours	53.33	53.33

LSD-value-of-larvae-for-hours-(H)-=-20.78
LSD value of adult for hours (H) = 18.95

The maximum mean percent mortality of *Tribolium castaneum* larvae was found after the exposure time of 96 hours with the mean mortality of 53.33% in both larvae and adults. After exposure of 72 hours the mortality was 44.00% in larvae and 38.67% in adult. Average mean mortality was 34.67% in both larvae and adults was noticed after 46 hours. Minimum mean mortality of 24.00% in larvae and 26.67% in adults was observed after the exposure of 24 hours (Fig. 4; Table 4).

Discussion

The extracts of four plants Peppermint (*Mentha piperita*), Thyme (*Thymus vulgaris*), Rosemary (*Rosmarinus officinalis*) and Lemon blam (*Melissa officinalis)* were used against *Trogoderma granaium* and *Tribolium castaneum* in the present study. There were several researches done on this literature where plant extracts were used against *Trogoderma granarium* and *Tribolium castaneum.* The result of some earlier research work may be analogous with the present

discovery. In the present study among all plant extracts *Rosmarinus officinalis* are most efficient extract against the larvae and adults of both pests. Laznik *et al.,* (2012) also reported that the essential oil of *Rosmarinus officinalis* was proved to be the most efficient fumigant, against another pest *Sitophilus granarius* (granary weevil) adults, caused more than 60% mortality of this stored pest, which indicates that *Rosmarinus officinalis* is an effective agent against coleopterans. However, *Mentha piperita* gave satisfactory efficacy of about 97% but only at high temperature. Jabr (2006) investigated the effect of seven plant essential oils against *Oryzaephilus surinamensis* and *Tribolium castaneum,* and reported that *R. afficinalis* was the least toxic to both insect species that caused 17.1% mortality in *Tribolium castaneum,* its mean that *Rosmarinus officinalis* is not efficient against *Tribolium castaneum*. Omer *et al.,* (2012) evaluated the effect of four plants extracts *Quercus infectoria*, *Solanum nigrum*, *Xanthium strumarium* and *Datura stramonium* against the mortality of *Trogoderma granarium*. Another study reported that *Rosmarinus officinalis* had significant effect on the mortality rate against another member of coleopteran *Tribolium castaneum*, however, thyme reported lowest mortality rate against *Tribolium castaneum* (Clemente *et al.,* 2003). Rozman *et al.,* (2007) reported that no oil compounds of *Rosmarinus officinalis* achieved more than 20% mortality after exposure of 24 hours, even with the highest dose against *Tribolium castaneum*. However, *Mentha piperita* showed second highest mean mortality against larvae and adult of *Trogoderma granarium*. Shaaya *et al.,* (2009) conducted a study in which different plant extracts were used out of which thyme caused 60% mortality rate on *Trogoderma granarium* larvae.

Conclusion

It is recommended that botanical extracts is highly efficient for controlling stored grain pests. In this present study these extracts proved to be a valuable method for controlling *Trogoderma granarium* and *Tribolium castaneum*. This biological method for controlling stored grain pest is an efficient technique as compare to chemical method because of their high toxicity that affects the quality of crop are very costly and are not affordable. In this study by using these plants extracts which included peppermint (*Mentha piperita*), Thyme (*Thymus Vulgaris*), Rosemary (*Rosmarinus officinalis*) and Lemon blam (*Melissa officinalis)* mortality rate of khapra beetle and red flour beetle was determined. It is recommended that other plant extract should be used for controlling other stored grain pests. It is concluded that the application of these medicinal plants may be promising in protecting the stored products against insect pests without hazardous effects.

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