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Insecticidal Efficacy and Repellency of Trans-Anethole Against Four Stored-Product Insect Pests

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ABSTRACT
In this study, it was investigated that repellency and insecticidal efficacy of trans-anethole of botanical origin on major stored product on pests species, namely *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Tribolium confusum* Jacquelin du Val (Coleoptera, Tenebrionidae) and *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). Repellency effect was evaluated by choice test in petri dishes, while the mortality effect was examined by exposure to wheat treated at different concentrations, such as 1, 2, 3, 4, and 8 µL trans anethole. In efficacy tests, after 72 hours exposure, the highest adult mortality was found on *T. castaneum* with a 60% mortality, while other test species showed no significant mortality. On the other hand, repellency tests revealed varying degree of repellency depending on the application dose of trans-anethole. It was determined that *S. granarius* belongs to repellent class III, while all of the other species fall under repellent class IV. F1 progeny decreased as trans-anethole concentration increased, and in this context 8 µL of trans-anethole is proved to be the optimal concentration causing maximum decrease in progeny production. Among the insect species tested, *T. confusum* was found to be the most sensitive to trans-anethole with 100% decrease in F1 progeny production. Our results indicate that trans-anethole can be used as a potential repellent for the control of major stored grain pests. Additionally, trans-anethole, by its contact efficacy, might be considered as a grain protectant against *S. granarius, S. oryzae, T. confusum* and *T. castaneum*.

Keywords: Apiaceae; Plant essential oil; Repellency; Progeny; Stored product insect

1. Introduction

Stored grains are often subjected to quality and quantity losses of varying magnitude during the storage (Boxall 2001; Ferizli & Emekci 2010). Grain deterioration which caused by several biotic and abiotic factors can be occurred in various ways, such as germination, clumping, self-heating, burning, baking quality, color and many others (Amruta et al 2015). Among the biotic factors in stored grain, insects stand as the most important cause of deterioration that result in significant economic losses (Boxall 2001). Post-harvest loss of grains caused by insect pests can be prevented or minimized through careful stored grain management. The most common and effective method against stored products pests is fumigation (Shaaya & Kostyukovsky 2011). Synthetic fumigants such as methyl bromide, aluminum phosphide, sulphuryl fluoride, carbonyl sulphide, ethane nitrile, and ethyl format are currently being used in pest control (Bond

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1984; Taylor 1994; Villers et al 2010; Mutungi et al 2014). These chemicals, however, can pose risks to environment and human health, as in the example of methyl bromide, which is currently prohibited by the Montreal Protocol due to its ozone depletion potential. European Pesticide Regulation [(EC) No. 1107/200] is another restrictive policy instrument in effect for the prevention of hazardous chemical use. Therefore, there is a big demand for novel pest control approaches using less toxic substances. Among the alternative approaches, secondary plant metabolites has gained much attention (Taiz & Zeiger 2002).

Secondary plant metabolites have behavioral and biological effects on insects are categorized in various classes (Güncan & Durmuşoğlu 2004). The most effective compounds against insect pests are alkaloids, glycosides, phenols, terpenoids, tannins, and saponins (Shanker & Solanki 2000). These compounds can play important roles in plant defense mechanisms against insects, such as toxicity, feeding cessation, repellency, locating prey or hosts by predators and parasitoids. Trans-anethole is a secondary metabolite which is synthesized from plants belonging to Apiaceae family, in particular. Bio-efficacy of plant essential oils and their major components against stored product insect pests are very well documented (Hikal et al 2017). Essential oils do not cause environmental pollution, do not leave residues unlike synthetic toxic chemicals, and are not dangerous to non-target organisms in nature (Regnault-Roger et al 2012). Moreover, fumigant activity of trans-anethole has been proven against major stored product insects in several studies (Shaaya et al 1991; Ho 2000; Mondal & Khalequzzaman 2010). For these reason essential oils can be promising materials in the development of alternative plant protection products against stored product pests (Shaaya et al 1991; Shaaya et al 1993; Ho 2000; Huang et al 2000; Wang et al 2001; Karakoç et al 2006; Mondal & Khalequzzaman 2010; Pimentel et al 2010).

In this study, insecticidal efficacy and repellency of trans-anethole against four major insect pests of stored products were investigated. In addition, inhibitory effect of trans-anethole on progeny production of the same insect species was also evaluated.

2. Material and Methods

2.1. Insect rearing

All of the test insects were obtained from laboratory colonies maintained continuously at the stored product pest laboratory of Directorate of Plant Protection Central Research Institute (Ankara, Turkey) since 2008. A rearing medium composed of a mixture of crushed soft wheat (*Tritucum aestivum* L.) and powdered dry yeast (*Saccharomyces cerevisiae* Hansen) (20:1 w/w) was used to rear *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) and *T. confusum* Jacquelin du Val (Coleoptera, Tenebrionidae). For rearing *Sitophilus granarius* and *S. oryzae* (L.) (Coleoptera: Curculionidae) whole soft wheat kernels were used (Ertürk et al 2017).

2.2. Contact toxicity of trans-anethole on grains

Experiments were conducted according to the standards given in Ndomo et al (2008). Five different doses of 1, 2, 3, 4, and 8 μL of trans-anethole (CAS number: 4180-23-8, 99%) were mixed with 1 mL of acetone and then each mixture was applied into 10 g wheat by using pipette and stirred with glass rod to ensure well mixture. In the control group, only 1 mL of acetone was applied to 10 g wheat. Treated wheat with trans-anethole solution at different doses was put under a fume cupboard for 5 minutes to evaporate the solvent and then transferred into jars, each of which contained 25 (one-day old) adult individuals of each species separately. The jars were kept in a climate chamber at 25±2 °C and 65% relative humidity (r.h.). The treatments were arranged randomized design with four replicates, each of which included the control group. After setting up the experiments, mortality counts for adults were made daily after treatment during following three days.

2.3. Effect of trans-anethole on F1 progeny

At the end of three days of contact toxicity tests, dead and live insects were removed from the treated wheat and the jars containing treated wheat only were retained at the same conditions for 60 days and at the end of 60 days F1 progeny adults were counted.

Inhibition rate was calculated by following formula.

$$IR = \frac{Cn - Tn}{Cn} * 100$$

Where; %IR, inhibition rate; *C*n, number of newly emerged adults in untreated (control); *T*n, number of newly emerged insects in treated replicates.

2.4. Repellent effect of trans-anethole

McDonald et al (1970)'s procedure was followed for the repellent activity of trans-anethole. Accordingly, 9 cm diameter discs of Whatman No. 1 filter paper was used. One half of each disc was treated with 1, 2, 3, 4, or 8 µL of trans-anethole in 1 mL of acetone, while the other half treated by acetone only. After aerating filter paper discs for 5 minutes to evaporate solvent, 20 (one-day old) adult individuals of *T. castaneum or S. granarius* in each concentration were separately placed on filter paper discs in Petri dishes. The inner sides of Petri dishes were coated with Fluon®PTFE (AGC Chemicals Europe, Ltd) to prevent the insects escaping. The covers of the petri dishes were drilled 1 mm in diameter to ensure air circulation. Petri dishes then incubated for 2 h at 25±2 ºC in the dark. At the end of 2 h, Petri dishes were opened and insects were counted according to which half of the paper discs they were settled on.

Percentage repellency was calculated according to the formula below:

$$PR = \frac{Nc - Nt}{Nc + Nt} * 100$$

Where; PR, percentage repellency value; Nc, number of insects in untreated (control) group; Nt, number of insects in treated group

Average percentage repellency values were categorized according to 0-V scale of Juliana & Su (1983). [Class 0 (PR< 0.1%); Class I (PR= 0.1-20%); Class II (PR= 20-40%); Class III (PR= 40.1-60%); Class IV (PR= 60.1-80%); Class V (PR= 80.1-100%)]

2.5. Statistical analysis

Prior the statistical analyses, percent mortality data obtained from the toxicity and repellency tests were transformed using arcsine transformation. The transformed data were then subjected to analysis of variance (ANOVA) followed by Tukey's multiple comparison test (P<0.05) using MINITAB® Release 16 package program.

3. Results and Discussion

3.1. Contact toxicity of trans-anethole on grains

Each species showed different levels of susceptibility to trans-anethole (Figure 1). *Tribolium castaneum* was the most sensitive species to essential oil with 60.16% mortality when exposed to trans-anethole at 8 µL for 72 h. Statistical analysis revealed that there were significant differences between doses ($F_{(4,15)}$= 307.87; P<0.05) Insecticidal efficacy of trans-anethole against *T. castaneum* increased with dose increases. The higher doses of trans anethole was not used for experiments, because of the odor residue problems on the treated product. The essential oil did not show any contact toxicity to *T. confusum* at any concentration rate tested throughout the exposure period. Toxicity effect on *S. oryzae* was also negligible with 8.67% mortality, at most, after 72 hours at the highest level of dosage ($F_{(4,15)}$= 21.96; P<0.05) (Figure 1).

Residual efficacy of plant essential oils as grain protectants was received less attention. In scientific arena, plant essential oils were rather used to evaluate their fumigant efficacy or contact toxicity by topical applications. Therefore, in this study, residual toxicity of trans-anethole against major stored-product insects on treated wheat grain were studied. Although no significant effect was obtained against test species except *T. castaneum,* our findings are contradictory with the previous studies (Karakoç et al 2006; Alkan & Gökçe 2012).

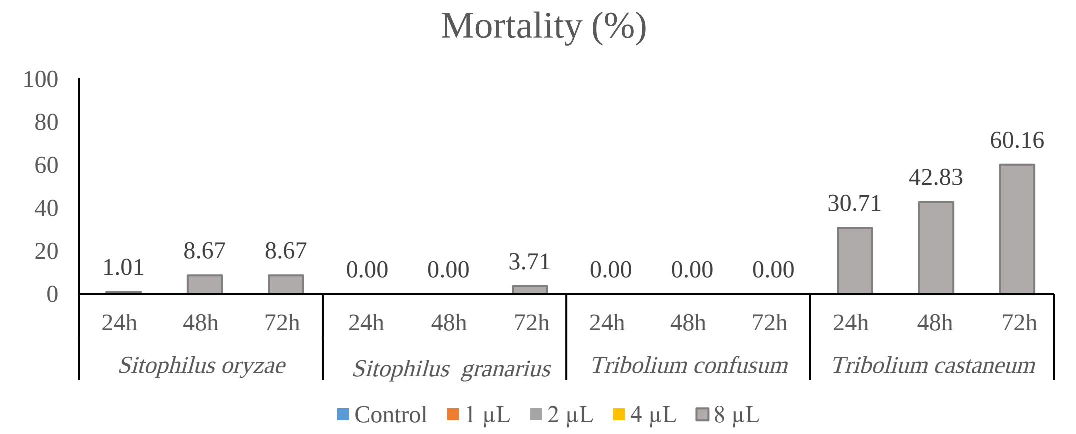

Figure 1- Mortality data of *S. granarius, S. oryzae, T. confusum* and *T. castaneum* exposed to wheat treated with trans-anethole at different rates (%)

3.2. F1 progeny assessment

F1 progeny suppression of test insects was increased with the application dose increased (Figure 2 and 3). The highest suppression rate was observed in *T. confusum* with a 100% decrease in F1 progeny at 8 µL of trans-anethole. Suppression rates in other species used in the experiments were as follows: *T. castaneum* (97.80%); *S. oryzae* (94.19%); and *S. granarius* (80%). Overall, the desired level of F1 progeny suppression activity was reached at the concentration of 8 µL. Tapondjou et al (2002) determined that, *S. granarius* was exposed for 48 h to 0.8% or 6.4% of the dry ground leaves *Chenepodium ambrosioides* L. (Chenepodiaceae) as grain protectant. Both low and high doses of *C. ambrosioides* completely suppressed in treated whole wheat against progeny production of *S. granarius*. Tapondjou et al (2005), showed that the F1 production of *S. zeamais* was completely suppressed on grains treated with *Eucalyptus saligna* and *Cupressus sempervirens* L. (Cupressaceae) crude oil extracts at the doses of 75 and 100 µL/40 g grain respectively. Contrary to other studies, it is believed that the 8 µL dose which provides 100% mortality in *T. confusum*, is due to the impurity of the crude essential oil composition used in other studies.

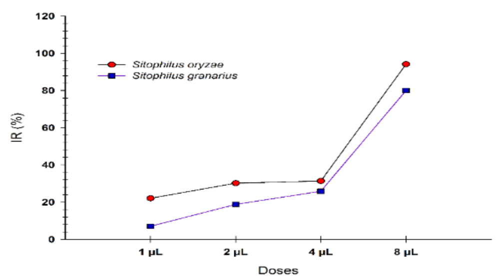

Figure 2- F1 progeny effect of Trans-anethole anetholeon *S. granarius* and *S. oryzae*

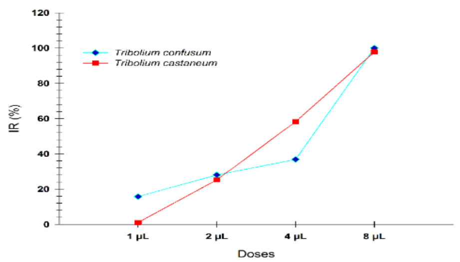

Figure 3- F1 progeny effect of Trans- on *T. confusum* and *T. castaneum*

3.3. Repellent effect of trans-anethole

Percentage repellency values increased as the concentration of trans-anethole increased (Table 1). PRs of four species at the lowest dose of 1 µL and at the highest dose of 8 µL of trans-anethole were, respectively, as follows: *S. granarius*, 12.5% and 97.5% ($F_{(3,15)}$= 175.0; P<0.05); *S. oryzae*, 47.5% and 95% ($F_{(3,15)}$= 26.9; P<0.05); *T. confusum*, 30% and 95% ($F_{(3,15)}$= 25.33; P<0.05); *T. castaneum*, 5% and 100% ($F_{(3,15)}$= 59.9; P<0.05).

Table 1- Repellency effects of Trans-anethole on test adults exposed to filter paper with four different concentrations for 2 h

	Repellency (%) ±SEM			
	Sitophilus granarius	*Sitophilus oryzae*	*Tribolium confusum*	*Tribolium castaneum*
1 µL	12.5±2.5c[1]	47.5±4.8c	30.0±5.8b	5.0±2.1b
2 µL	17.5±4.8c	57.5±2.5bc	80.0±8.2a	80.0±8.2a
4 µL	77.5±2.5b	65.0±2.9b	95.0±5.0a	90.0±5.8a
8 µL	97.5±2.5a	95.0±5.0a	95.0±5.0a	100.0±0.0a
Average	51.3±9.7	66.3±4.9	75.0±7.4	68.8±9.9
Repellency classes	III	IV	IV	IV

[1], Different letters in the same column indicate statistically different from each other (Anova P<0.05, Tukey test)

Essential oils and their compounds were previously reported having potent repellency activity on stored-product insects (Liu & Ho 1999; Isman 2000; Papachristos & Stamopoulos 2002; Garcia et al 2005). Amer & Mehlhorn (2006) emphasized the importance of studies with essential oils to unearth their repellency potential. In this regard, all of the four insect species we tested displayed a high sensitivity to trans-anethole and revealed high PRs. However, in terms of the lowest dose yielding the highest efficacy, *T. confusum* showed the highest sensitivity rate with 95% PR at 4 µL of trans-anethole. Our study should be considered as a preliminary attempt to reveal the potential of trans anethole to be used as a biorational pesticide. Moreover, further researches on such as slow release, encapsulation, and formulation of essential oils and their compounds are crucial to enhance their chance in the development of sustainable and cost-effective plant protection products.

4. Conclusions

The results of this study clearly demonstrate that trans-anethole with its efficacy in population suppression and its repellency, in particular, has a strong potential as a bio-pesticide against stored product insect pests. From environmental protection point of view it has also a high potential of use in organic farming, since trans-anethole is thought to be less harmful to environment. It can also be concluded that essential oils have broad spectrum pesticide activity due to the presence of several active ingredients that works with various mode of action than synthetic pesticides. Trans-anethole is found naturally in many plant species of Apiaceae, namely anise, fennel and star anise. There are not many alternatives to synthetic pesticides in controlling stored product pests. Therefore, trans-anethole, one of the few promising alternatives, is very important for controlling storage pests. However, in order to use Trans-anethole in field applications, additional studies on slow-release formulations and on residual remains on treated grains and on other surfaces should be undertaken.

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