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Oviposition deterrent and egg hatchability suppression of *Tilia cordata* (Tiliaceae) flower extract on *Sitophilus granarius* (Coleoptera: Curculionidae)

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Abstract
The efficacy of oil extracted from the flowers of *Tilia cordata* was evaluated in the laboratory against *Sitophilus granarius* infesting stored wheat grains. Flower extracts from *T. cordata* were obtained though the soxhlet extraction method using methanol and n-hexane as the solvent. Each of the extracts was tested by exposing six pairs of adult beetles to various levels of 0.5, 1.0, 2.0 and 4.0 ml corresponding to 3.0, 6.0, 9.0 and 12.0% v/w concentrations admixed with 25g wheat grains in three replications respectively. Control group treatment was s*et al*ong. The results showed that oviposition and percentage egg hatched were significantly (P <0.05) suppressed on seeds treated with higher treatment level of extracts. Flower extract with n-hexane at 4.0 ml (12.0% v/w) 25g wheat grains was most effective in suppressing oviposition and egg hatched. Therefore, *T. cordata* exhibit promising degree of oviposition deterrent and ovicidal properties and thus, have a great potential for use as a plant-based bio-insecticide as an alternative to synthetic insecticides for controlling *S. granarius* infestation on stored wheat grains.

Keywords: *Sitophilus granarius*, the wheat weevil, ovicidal effect, bioinsecticide, deterrent

Introduction

The wheat weevil, *Sitophilus granarius* Linnaeus, 1758 (Coleoptera: Curculionidae) is one of the most serious pests of stored grain products, especially in temperate regions (Niewiada *et al*., 2005). This pest is internal feeder and cause considerable loss to cereals affecting the quantity as well as quality of the stored grains (Ebadollahi, 2011; Karakas, 2017) [1, 2].

Infestation control of stored grain insect pests has relied mainly on the use of synthetic chemical insecticides such as methyl bromide and phosphine. The high supply costs associated with the continuous use of these chemical insecticides, the pest resurgence and resistance, the accumulation of toxic residues in food, the risks of contamination by the user, the impact on both human and environmental health have necessitated the search for alternative insect infestation control tools that are non-toxic and environmentally friendly (Ileke *et al*., 2014) [3].

The present study is to evaluate the efficacy of organic solvent extract of the linden, *Tilia cordata* (Tiliaceae) as a protectant for stored wheat seeds against *S. granarius* infestation.

Materials and Methods

Insect culture technique

The wheat weevil was feed on soft wheat seeds in the glass jars (1 L) having high moisture content so that *S. granarius* easily laid eggs and show better development. Soft wheat seeds were obtained from a local market of Ankara. A possible contamination effect was prevented by keeping the wheat seeds at -20 degrees for 3 days before using the insect culture. The wheat weevil was reared at controlled temperature 26 ± 2 ºC and relative humidity in the range of 65 ± 5% RH which is the best condition for its development. The dark (D) and light (L) periods (12h: D – 12h: L) were maintained according to best development of weevil because *S. granarius* is very sensitive to the light periods for meeting and proper development. After 3 days, the adult weevils were removed to obtain a homogeneous stock of adult weevil and this process was repeated for the next three generations. The homogeneous population is preserved for further experiments.

Test plant

In this study, bio-insecticide effects of flower extract from linden, *Tilia cordata* Miller (Tiliaceae) was tested against the wheat weevil, *S. granarius*. This medicinal plant was obtained from an herbal shop of Ankara. The dried flowers were milled into powder using a hammer mill. Thereafter, the milled flower powder of *T. cordata* was taken to the laboratory for extraction using methanol and n-hexane as organic solvent using soxhlet apparatus.

Soxhlet application

The plant material was extracted using the Soxhlet extraction method (Sharma and Gupta, 2009) [4]. A known amount (450g) of *T. cordata* was filled into the Soxhlet apparatus. A cotton plug was used at the place of thimble to stop the entry of the crude material into the siphoning tube. The required solvent was filled up five times more than the total amount of the sample material into the flask of the apparatus. The apparatus was then connected with the water supply to the condenser. The temperature of the heating mantle was maintained at 60-65 ºC. The process was carried out for 6 to 7 hours for each sample. The flower extracts were later concentrated using a rotary evaporator to obtain a concentrated extract, which was stored in a glass balloon for future applications.

Oviposition and fecundity tests

Tilia cordata, extracted at four dosages, namely 0.5, 1.0, 2.0 and 4.0 ml (corresponding to 3.0, 6.0, 9.0 and 12.0% w/v concentration) was measured into 9.0 cm diameter Petri dishes containing 25g of disinfected wheat seeds weighed using a digital weigh balance (model Ideen Welt Großes

LC-Display) in triplicates using a syringe and each is thoroughly mixed using a glass rod to ensure the uniform mixing of the extract with the grains and is left open to dry. Thereafter, six paired sexed adult insect of 7 days old *S. granarius* were introduced into each Petri dish containing different dosages of plant flower extract food complex of treated and untreated grains. The sex of *S. granarius* was determined by the pattern of Dinuta *et al*., (2008) [5]. There was also a control treatment that does not involve any addition of extract on the seeds. The Petri dishes were then covered to prevent insects from escaping. The number of eggs laid by the female beetles in the seeds was recorded on the 7th day after the introduction of beetles to seeds; this was used to calculate the percentage of egg hatching and the percentage reduction of egg lying, respectively as follows:

Percentage reduction of eggs laid:
No of eggs laid in control – no of eggs laid on treated grains / no of eggs laid in control x 100/1

Egg hatching (%):
No of eggs hatched / no of eggs in each Petri dish x 100/1

Statistical data
Data from the three replicates of the experiment were pooled together and subjected to one-way Analysis of Variances (ANOVA). Treatment means were separated using Least Significant Differences (LSD) at 5% probability level.

Results

Effect of *T. cordata* flower extracts on oviposition and fecundity
The effect of the flower extracts on oviposition is showed in Table 1. According the results, the extracts at all application levels significantly inhibited the female *S. granarius* from laying eggs on treated wheat seeds. In spite of the early death of *S. granarius* adults, no concentration of the flower extracts could completely prevent the females from oviposition. The percentage reduction in the number of the laid eggs was inversely proportional to the extracts concentration tested. The laying capacity gradually decreased with the increase in the treatment dose of each extract. The maximum reduction in egg laying was noticed with n-hexane extract 48.95% reduction on wheat grains treated with 4 ml dosage rate as against 20.73% recorded in control group. The same trend was recorded in methanol extract when at 4 ml, 48.55% eggs reduction was observed on treated wheat seeds against 27.52% in control group treatment. Statistically, there was a significant difference between the tested concentrations compared to control group.

Table 1: Mean percentage reduction in number of eggs laid by female *Sitophilus granarius*

Treatments 25g wheat seed	Reduction in eggs laid (%)	
Concentration (ml / %)	**Methanol**	**n-Hexane**
0.0 / 0.0*	27.52 ± 3.3	20.73 ± 1.4
0.5 / 3.0	36.94 ± 3.1	24.53 ± 1.8
1.0 / 6.0	44.65 ± 3.6	26.57 ± 2.6
2.0 / 9.0	44.97 ± 2.3	38.12 ± 3.2
4.0 / 12.0	48.55 ± 4.1	48.95 ± 1.7
LSD (5%)	9.07	20.44

*Control group

Effect of *T. cordata* flower extracts on egg hatching
In the present research, the effect of *T. cordata* flower extracts on egg hatching capability of *S. granarius* revealed that there was a significant reduction. The egg hatching capacity gradually decreased with the increase in the treatment dose level of each flower extract. The maximum reduction in the hatched eggs was noticed at 4 ml (12%) level when only 5.91% and 6.15% eggs found to be laid, hatched on the seeds treated with n-hexane and methanol extracts, respectively as against 29.86% and 29.27% hatched eggs in control group (Table 2). The results revealed that *T. cordata* flower extracts, at different dose level, were very effective against *S. granarius* egg viability on stored wheat seeds.

Table 2: Mean percentage of egg hatching of *Sitophilus granarius* from treated wheat seeds

Treatments 25g wheat seed	Egg hatching (%)	
Concentration (ml / %)	**Methanol**	**n-Hexane**
0.0 / 0.0*	29.27 ± 3.9	29.86 ± 2.7
0.5 / 3.0	18.62 ±3.1	13.96 ± 2.1
1.0 / 6.0	10.66 ± 1.8	10.26 ± 1.4
2.0 / 9.0	7.36 ± 0.6	8.87 ± 1.2
4.0 / 12.0	6.15 ± 1.8	5.91 ± 0.7
LSD (5%)	6.95	2.87

*Control group

Discussion
The results of the study provide empirical evidence that the insecticidal activity of *T. cordata* flower extract with methanol and n-hexane can be effective to varying degrees in deterring oviposition and egg viability by *S. granarius*, and ultimately reduced the percentage seed damage and weight loss due to infestation by the foregoing insect pest. However, no concentration of plant extracts completely prevented females from laying eggs on seeds. This research also showed that plant extracts significantly reduced the number of eggs laid per female compared to that obtained in the control.
The marked decrease in egg laying was perhaps the result of the slight suppressive effect these volatiles exert on the mating of legume beetles, a determining factor in influencing the insect's subsequent egg count (Engelmann, 1970) [6]. Current findings support the observed observation for oil vapours on other beetle, *Callosobruchus maculatus* (Paranagama *et al*., 2003) [7]. These findings were reported by Elhang (2000) [8], Kim *et al*. (2003) [9], Ghoswal *et al*. (2004) [10] and Abdullahi (2011) [11] noticed that the reduction in legume beetle egg laying was significantly higher when seeds were treated with various pesticide plant extracts, and there was a similar trend for some vegetable oils. The reduction in oviposition in seeds treated with the extract may also be due to an extract film on the seeds that is unsuitable for oviposition. The results revealed that various pesticide plants at different dose levels acted as the highest ovipositional deterrents. The ability of the extract to reduce the egg-laying capacity of female insects can be attributed to the presence of flavonoids in the plant (Zabri *et al*., 2008; Zabri *et al*., 2009) [12, 13]. This confirmed the findings of Righi-Assia *et al*. (2010) [14] that flavonoids significantly reduce ovulation and fertility in *Calyptraea chinensis*. The results from this study equally show that the plant extract can interfere with normal embryonic development by suppressing hormonal and biochemical

processes. A similar physiological conclusion was made by Ofuya *et al*. (1992) [15], Jayakumar *et al*. (2003) [16] and Raja and William, (2008) [17].

The flower extract used in this study was found to be significantly superior in reducing hatching; as the concentrations of the extracts increased, their inhibitory effects on hatching of eggs also increased. Egg mortality and failure to hatch in extract-treated seeds have probably been attributed to the toxic component of the extracts, as well as physical properties that cause changes in surface tension and oxygen tension in eggs (Singh *et al*., 1978) [18]. The ovicidal effect of the extracts on bruchid can also be explained as suffocation by blocking the main gas exchange pathway between a thin area of the chorion and the outside (Credland, 1992) [19], which ultimately reduced the emergence of insects from the external environment processed seed (Copping and Menn, 2000) [20].

Conclusion

Aromatic plants have a variety of chemicals that can be isolated and used for the control of stored grain product beetles. These aromatic plants will produce environmentally sensitive chemicals that have no harmful effects on non-target organisms. The findings of alternative insecticides will allow their use in rotation programs, which can lead to increasing efficiency in insect control as well as minimizing the development of resistance. The potential use as an insecticide of plant compounds such as secondary metabolites, essential oils, peptides and entomopathogenic extracts from different parts of the plant are recommended as bioinsecticides.

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