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Antifeedant Activity of the Essential Oils from Four Different Lamiaceae Species against *Agelastica alni* L. (Coleoptera: Chrysomelidae)

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Abstract Phytochemicals have long been touted as alternatives to synthetic chemicals for pest control and especially interest has grown for the search of antifeedants effects over the last decades. Similarly, we tested antifeedant activity of essential oils obtained from four different Lamiaceae species; *Thymus transcaucasicus* RONNIGER, *Thymus pseudopulegioides* KLOKOV and DES.-SHOST, *Thymus leucotrichus* HAL. and *Teucrium polium* L., against *Agelastica alni* L., Coleoptera: Chrysomelidae larvae. *Thymus leucotrichus* showed the most powerful antifeedant effect of the four plant species during the three days ($r = 0.481$, $P < 0.01$) the highest AFI value (AFI = 41.055) was obtained on the second day at 2000 ppm ($P < 0.05$). At the same time, the first and third day's treatments showed strong positive correlation between dose and AFI indices [1^{th} day; $r = 0.890$, $P < 0.01$ and 3^{th} day; $r = 0.918$, $P < 0.01$]. According to these results, *Thymus leucotrichus* essential oil is the most effective phytochemical against *A. alni* larvae.

Keywords Antifeedant, Pest, *Agelastica alni*, Lamiaceae, Phytochemicals, Essential Oil, Biological Control

1. Introduction

Agelastica alni L., Coleoptera: Chrysomelidae (alder leaf beetle) is one of the most serious pests of hazelnut and alder trees all over the world, especially in Turkey (Suchy, 1988; Baur et al., 1991; Urban, 1999). This pest is mostly controlled with chemical pesticides like; carbaryl, methiocarb and malathion. However, these chemicals affect environment and untargeted organism negatively and these effects have led to new approaches like biological control methods for controlling this pest (Tomalak, 2004; Sezen et al., 2004).

Plants are alternative sources for controlling pest and the use of natural plant products as pest controlling agent is gaining importance in recent years. When purified phytochemicals or crude plant extracts are used on insects, their effects show in several ways; toxicity (Hiremath et al., 1997), growth retardation (Breuer and Schmidt, 1995), feeding inhibition (Klepzig and Schlyter, 1999; Wheeler and Isman, 2001), oviposition deterrence (Dimock and Renwick, 1991; Hermawan et al., 1994; Zhao et al., 1998), suppression of calling behaviour (Khan and Saxena, 1986) and reduction of fecundity and fertility (El-Ibrashy, 1974; Muthukrishnan and Pushpalatha, 2001). In addition, these compounds are safe for advanced animals and environment; furthermore, they do not contribute to resistance development or pest resurgence (Prakash and Rao, 1997; Talukder and Howse, 1994).

The aim of the this study, which appeared with all of this information, was to evaluate the antifeedant properties of four different plants essential oils; *Thymus transcaucasicus* RONNIGER, *Thymus pseudopulegioides* KLOKOV and DES.-SHOST, *Thymus leucotrichus* HAL. and *Teucrium polium* L., against *Agelastica alni* L. larvae.

2. Materials and Methods

Collection of Test Insects

Larvae of *A. alni* were collected from the vicinity of Trabzon, Turkey, during April to June in 2013. When the larvae were taken to the laboratory, 3^{th} instar larvae were separated and placed in plastic boxes for experiment.

Plant Materials

Plant materials were collected during 2012 to 2013 from the vicinity of Trabzon, Giresun, Bayburt and Rize in Turkey. Fresh plant materials were air-dried in shade condition and fragmented. Essential oils were obtained from the following plants: *Thymus transcaucasicus, Thymus pseudopulegioides, Thymus leucotrichus* and *Teucrium polium* with Clevenger. Then each essential oil was weighed and stored in +4 °C until used.

Antifeedant Test

A no choice test was set up with 3 replicates for each test dose and replicated over 3 days. Each test group was set up with petri dishes (9 cm in diam.) each including 5 larvae (3^{th} instar) and wet filter paper in the center. One untreated control was also set up. Test dose were prepared from the stock essential oils by further dilution in 50% methanol in H_2O to produce five different concentrations: 250, 500, 1000, 2000, 4000 ppm. Leaf discs were prepared from alder tree leaves using a cork borer and weighed before the test. Each leaf disc was immersed in the test solution while fully covered. In addition, control leaf discs were immersed in 50% methanol in H_2O solution and all discs were left at room temperature for 3 min. to let the solvent evaporate. Then, each disc was placed in petri dishes and all test groups placed in growth chamber (BINDER KBWF720) (25 ±1°C and L16: D8 photoperiod). After 4 h, the remnants of leaf discs were collected and dried separately at room temperature to a constant weight. This process was repeated for each of the three treatment days. The amount of consumed food was calculated depending on the initial fresh weight of each disc and the dry weight of its remnants. The antifeedant index was calculated according to; $AFI = [(C – T) / (C + T)] \times 100$ formula (Sadek, 2003); C as the consumption of control discs and T the consumption of treated discs. The food consumed by the 5 larvae that were given control discs were averaged, and the means were used as C for the calculations of the AFI for each observed T.

Statistical Analysis

The antifeedant indices at different treatments were compared using an analysis of ANOVA followed by Duncan test for multiple-comparison where significant differences were observed. A Pearson correlation coefficient test was carried to determine the AFI-Day and AFI-Dose relations between the treatments. All these analysis performed with SPSS version 21.0 for Windows (Yang, 2014).

3. Results

In this study, end of the analysis and observation identified how effectively the all plant essential oils dissuaded *A. alni* larvae feeding. Antifeedant indices that calculated according to AFI formula, show differences from plant to plant. The determined results of the antifeedant activities according to dose and days relations are shown in Table 1. The most high AFI values had been observed second day for all treatments.

The highest AFI value was determined on the second day at 250 ppm dose (AFI = 45.326) and the lowest value was observed at 2000 ppm on the first day (AFI = 3.181) with treatment of *T. transcaucasicus* for all days (Fig. 1). There were significant differences between 250 ppm and the other doses of all days ($P < 0.05$). In addition, on the second day, there was a negative correlation between dose and AFI indices ($r = - 0.525$, $P < 0.05$).

For *T. leucotrichus,* the highest AFI value (AFI = 41.055) was obtained on the second day at 2000 ppm ($P < 0.05$), the lowest value (AFI = 13.823) was assigned on the first day at 250 ppm for all treatment days (Fig. 2). At the same time the first and third day's treatments showed strong positive correlation between dose and AFI indices [1^{th} day; $r = 0.890$, $P < 0.01$ and 3^{th} day; $r = 0.918$, $P < 0.01$] (Table 1). However, during the three days, the treatment of *T. leucotrichus* showed the most powerful antifeedant effect of the four plant species ($r = 0.481$, $P < 0.01$).

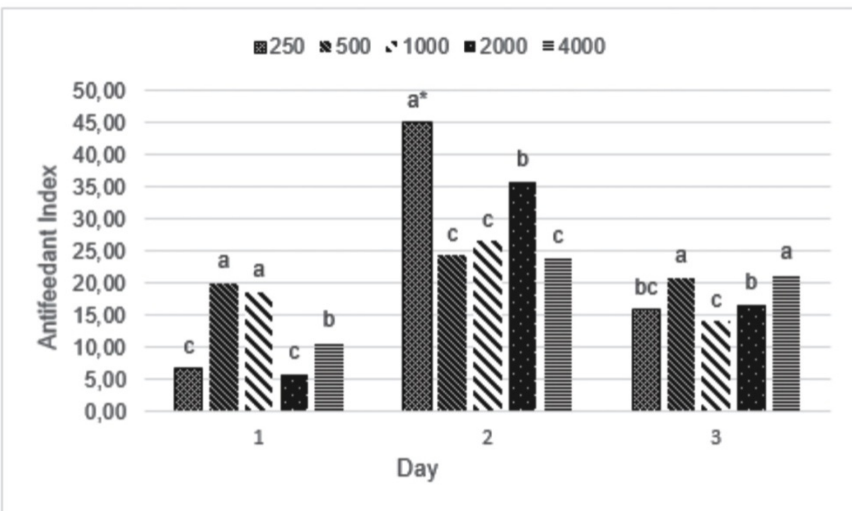


Figure 1. Effect of essential oil of *T. transcaucasicus* on *A. alni* larvae according to doses and days. * show the most effective AFI value between doses for all days

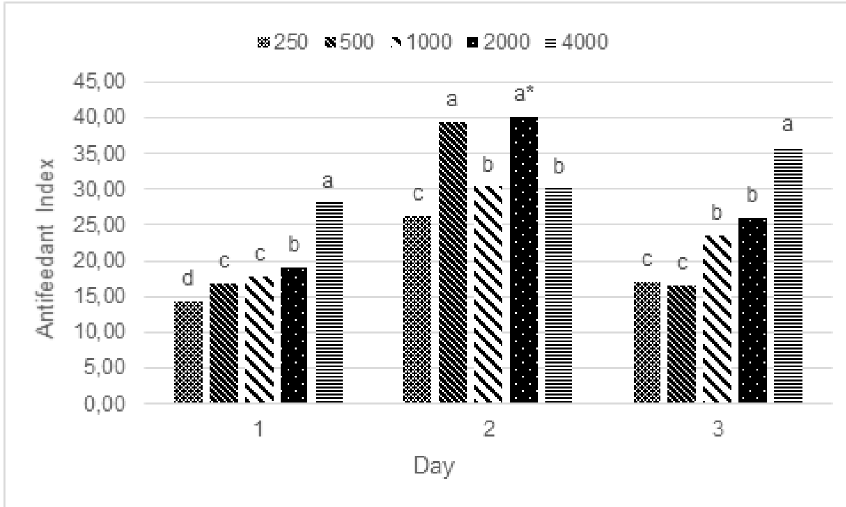
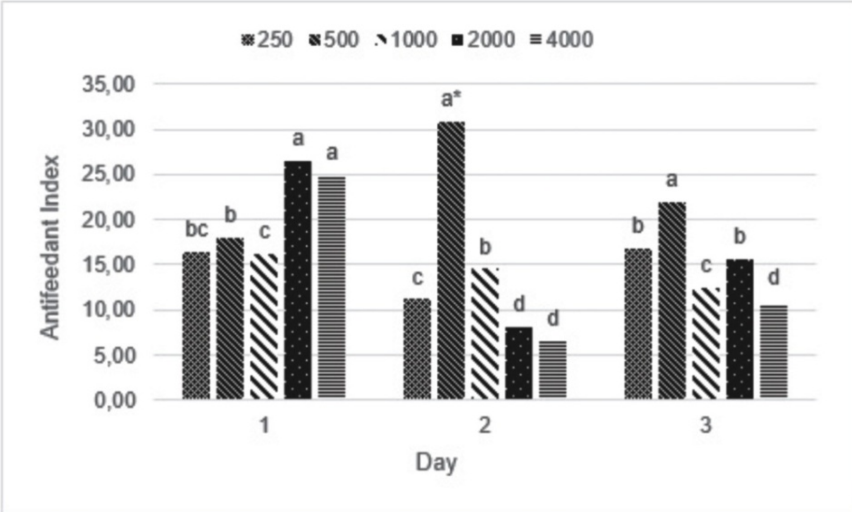


Figure 2. Effect of essential oil of *T. leucotrichus* on *A. alni* larvae according to doses and days. * show the most effective AFI value between doses for all days

Table 1. Pearson correlation coefficients between essential oils calculated for AFI indices of *A. alni*

	Thymus transcaucasicus			**Thymus pseudopulegioides**			**Thymus leucotrichus**			**Teucrium polium**		
	1th Day	2th Day	3th Day	1th Day	2th Day	3th Day	1th Day	2th Day	3th Day	1th Day	2th Day	3th Day
Thymus transcaucasicus	-0.151	-0.525*	0.117									
Thymus pseudopulegioides				0.201	-0.549*	0.852**						
Thymus leucotrichus							0.890**	0.209	0.918**			
Teucrium polium										0.805**	-0.516*	-0.669**
1th Day	1	-0.699**	0.117	1	0.176	0.391	1	-0.036	0.913**	1	-0.530*	-0.324
2th Day	-0.699**	1	-0.494	0.176	1	-0.516*	-0.036	1	-0.109	-0.530*	1	0.800**
3th Day	0.117	-0.494	1	0.391	-0.516*	1	0.913**	-0.109	1	-0.324	0.800**	1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)



* show the most effective AFI value between doses for all days

Figure 3. Effect of essential oil of *T. polium* on *A. alni* larvae according to doses and days.

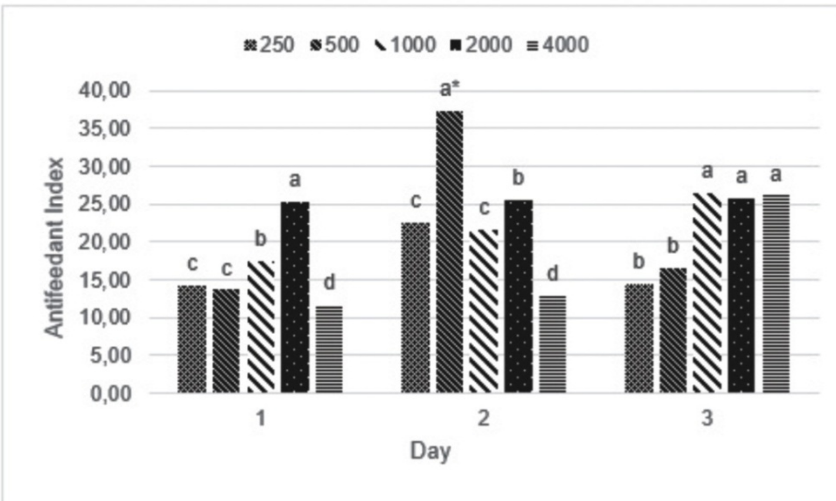


Figure 4. Effect of essential oil of *T. pseudopulegioides* on *A. alni* larvae according to doses and days. * show the most effective AFI value between doses for all days

On the other hand, *T. polium* had the weaket antifeedant effect against the *A. aln i* larvae according to all other tests that contain other plant species. On the second day, 500 ppm was the most effective dose in comparison to other doses (AFI = 31.521) (Fig. 3). Meanwhile, there was a strong positive correlation between dose and AFI values on the first day ($r = 0.805$, $P < 0.01$). But this situation was reversed completely on the second and third day (2th day; $r = -0.506$, $P < 0.05$ and 3th day; $r = -0.669$, $P < 0.01$).

T. pseudopulegioides had variable correlation between the dose and AFI indices. On the second day, AFI value reached the 37.896, which was the highest value for *T. pseudopulegioides* (500 ppm) (Fig. 4). While the third day's treatments showed strong positive correlation between dose and AFI indices ($r = 0.852$, $P < 0.01$), on the second day there was negative correlation ($r = -0.549$, $P < 0.05$).

4. Discussion

Phytochemicals have long been touted as alternatives to synthetic chemicals for pest control and especially interest has grown for the search of antifeedants effects over the last decades (Koul, 2008). Similarly we tested antifeedant effectiveness of essential oils obtained from four different plant species on *A. alni* larvae. All these plants are in the Lamiaceae family and this family is known for bioactive essential oils that have efficient insecticidal effect on insect pests (Regnault-Roger et al., 1993). But in this study, larval death was not observed during the three test days.

In literature, there are not enough studies about these species. There is no antifeedant study about *T. transcaucasicus, T. pseudopulegioides* and *T. leucotrichus* in literature and there are a few studies about *T. polium*. Heydarzade and Morawej (2012) reported that *T. polium* essential oil had toxic effects on *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) adults. But there is no detailed antifeedant study about the *A. alni.*

In our study, all test plants showed antifeedant effect on *A. alni.* However, this effect was not as high as expected from the plants before the study. This situation is thought to be relevant with insect biology and literature supports this view. *A. alni* is an oligophagous herbivour insect (Firidin and Mutlu, 2009). Thanks to this life strategy, it is exposed to different phytochemicals and it has adapted itself. Meanwhile, this situation helps insects to create resistance against the synthetic chemicals (Issa et al., 1984a, b). This was probably also the main reason why AFI indices, derived from results of no choice tests; remained in narrow scale. Additionally, it would be noted that in no-choice tests, the antifeedant activity was generally found lower than in the choice tests due to the accessibility of untreatment food (Sadek, 2003). From the results obtained in this study, it is suggested that future studies would be carried with choice and no-choice tests together.

Nowadays, a new approach is adopted for integrated pest management. According to this approach, an insecticides do not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001). So antifeedant properties of phytochemicals have gained a special importance to investigate. This is because pest damage can be reduced with these phytochemicals without killing the pest.

The following conclusion can be made from the results of this study. During the three days, the treatment of *Thymus leucotrichus* showed the most powerful antifeedant effect of the four plant species. And this species essential oil also has potential use with management of *Agelastica alni*.

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