Ethanol Extraction of *Hyoscyamus niger* and *Hypericum calycinum* Evaluated for Acaricidal Activity against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract: *Hyoscyamus niger* L. (Solanaceae) and *Hypericum calycinum* L. (Hypericaceae) were collected from two provinces Bursa and Ankara in Turkey during flowering stages. Extracts obtained from *H. niger* and *H. calycinum* were tested against two-spotted mite as an acaricidal pesticide. Bioassay experiments were carried out under laboratory conditions using two different methods namely, leaf disc dipping and direct leaf spraying. Two-spotted mite was reared in insectary using *Phaseolus vulgaris* L. (Fabaceae). In the leaf dipping method, *H. niger* extract resulted in an effective adjusted mortality for larvae of 87.13% and for adults of 81.66%. For *H. calycinum* the adjusted mortality for larvae and adults was 77.77% and 85.97% respectively at the highest concentration. In larval stage, LC₅₀ values were 5.47 w/v and 3.32 w/v dipping and spraying method respectively. In addition, adult stage, it was determined that LC₅₀ values were 1.17 w/v and 3.56 w/v in both methods. For leaf spraying method, *H. niger* resulted in an adjusted showed an effect against larvae and adults mortality rate was 95% and 91% respectively. However, *H. calycinum* mortality percentage was 80% and 87% at 12% concentration, with LC₅₀ values of 9.62 w/v and 2.13 w/v in dipping method. In this research apart from mortality effects on egg, reproduction was also investigated. Each concentration showed that treated females laid less eggs compared to the untreated females.

Key words: Two-spotted spider mite, henbane, St-John's Wort, extract, acaricidal effect.

1. Introduction

In general, agricultural systems as a whole are facing a variety of challenges. Among them insect pests (e.g. aphids, leafhoppers, white fly, bollworms, thrips, and mites) are the main factors causing serious damage for many cultivated crops in agricultural systems in many parts of the world including Turkey [1]. Two spotted mites [*Tetranychus urticae* Koch (Acari: Tetranychidae)] are a global pest on a variety of host plants including many field crops, ornamentals, weeds, and many native plants.

With the introduction of synthetic chemical

pesticides in the 1950s, pesticides became the most popular approach to control insect pests [2]. The search for safer and less harmful to humans and environment has stimulated the investigation of alternative pesticides such as plant extracts obtained from different medicinal plants.

Two-spotted spider mite is a cosmopolitan and a most important pest worldwide. It is one of the most damaging pests because of its broad host range and response to toxic plants, and has become a major pest on many crops, fruit trees, vegetable crops, cotton, ornamentals, and glasshouse crops [3]. The two-spotted spider mite has piercing and sucking mouthparts. The adults and immature stages cause damage to the host plants by piercing the plant cells

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and sucking out the sap. The damaged cells appear yellowish with stippled spots on the upper surface of the leaf. The mite generally feeds on the underside of the leaf, the surface of which the pest covers with a web. They have a short life cycle and favorable environmental situations cause populations of the pest to increase rapidly. The mites prefer hot, dry weather conditions, therefore severe mite feeding on the leaves causes damage to the epidermis, which accompanied by dryness, leads to leaves falling off. This also reduces the quality and quantity of the crop. According to Zhang [4], two-spotted spider mite has about 1,200 host plants of which more than 150 are economically important. The short life cycle and rapid population development and high fecundity of the females lead to a high risk of pest outbreaks. To control mite populations for many years conventional chemical control of mites has been extensively practiced. A result of indiscriminate use of conventional pesticides *T. urticae* has developed resistance to most of the conventional pesticides used to control it. Stumpf and Naven [5, 6] indicated that two-spotted spider mite has developed resistance to organophosphates, clofentezine and abamectin. According to Attia et al. [7] the mites have developed a resistance to more than 80 different acaricides. In addition, disadvantages of conventional insecticides, such as harmfulness to users, the environment and non-target species, and the natural enemies of the pests, means that there has been increasing interest in novel alternative insect pest control methods that are effective, selective and safe to humans and the environment [8]. Plants based on pesticides or bio-pesticides are one of the main alternatives to conventional insecticides. They can be used to control insect pests by acting as repellents, anti-feedants, oviposition deterrents, and by causing developmental disruptions, or even by killing the targeted pests [9].

Many research projects have been conducted in relation to effects of plant extracts against economically important insect pests [10, 11, 12]. For

example, *Hyoscyamus* spp., also known as “henbane” has been known for its medicinal effects, which also showed insecticidal effect against some arthropoda. However, henbane can also be toxic, even fatal, to animals in low doses, the larvae of some lepidoptera species, including cabbage moth, showed toxicity when they fed on [13]. It was revealed that the extract of *H. niger* produced the greatest contact toxicity on *Trialeurodes vaporariorum* (Westw.) (Hem.: Aleyrodidae) [14]. According to Erdogan and Yildirim [15] the extract obtained from *H. calycinum* was effective against *Myzus persicae* Sulzer (Hem.: Aphididae) as an insecticide. However, no research has been done for the acaricidal efficacy against two-spotted spider mites that has been published.

The aim of this research was to evaluate the acaricidal effects of ethanolic extracts obtained from *H. niger* and *H. calycinum* against two-spotted spider mite.

2. Materials and Methods

2.1 *Tetranychus urticae* Culture

T. urticae was reared on bean [*Phaseolus vulgaris* L. (Fabaceae)] plant under conditions of 25 ± 1 °C, $65 \pm 5\%$ relative humidity and 16 L:8 D photoperiods. Green bean plants were grown under pesticide free conditions in a glasshouse. Bean seeds were raised in plastic pots (15 cm diameter) containing potting mixture and slow release fertilizer and watered every second day. Bean plants were used as mite stocks and in the experiments were grown in a greenhouse. A glasshouse colony of two-spotted spider mites was established on the bean plant when they reached about six to eight leaf stage. Two-spotted spider mites were collected from the experimental field, the institute, and from cucumber plants. Infested leaves were cut off from the cucumber plants and placed on the bean plants growing in the glasshouse under conditions of 25 ± 1 °C, $65 \pm 5\%$ relative humidity and 16 L:8 D photoperiod. In another glasshouse, bay extra bean plants were raised for their leaves to be used in the

experiments.

2.2 Preparation of Plants and Crude Extraction

The plant materials used in this research were collected from two different provinces during 2008. *H. calycinum* was collected from Bursa province and *H. niger* was collected from Ankara province. Both species were collected while actively growing at flowering stage. All materials were harvested by cutting at soil level and the whole plants were used in extraction. The collected plant material was dried in room temperature ($25 \pm 1^\circ\text{C}$) under shade for several weeks. The dried plants were grinded into small particles by using an electric grinder. Dried plant material was prepared from each sample of 200 g from each powdered plant. Brauer and Devkota [16] described the method for extraction used in this study. The extraction procedure used was 200 g powdered sample from each plant, which was deposited into an Erlenmeyer flask with 500 mL of ethanol (80%) and left for 72 hours. A soxhlet apparatus was used for the extraction. Extraction was finished within 6 hours. To remove/evaporate ethanol from the crude extraction a rotary evaporator was used (50-60 $^\circ\text{C}$). The obtained crude extracts from each plant was then reconstituted at a concentration of 20% (W/V) using ethanol 80% (V/V in distilled water) as stock solutions and stored at 4 $^\circ\text{C}$ in colored glass vials. For the evaluation for efficacy tests, the crude materials for stock solution were dissolved in distilled water containing Triton X100 at a rate of 0.1 mL/L.

2.3 Effects of Obtained Plant Extracts on Two-Spotted Spider Mite

In bioassay experiments concentrations used were 1%, 3%, 6% and 12% plus untreated control (untreated). For these bioassays first instar larvae and three day old adults were used. Disposable petri dishes were used by using 3 cm diameter bean leaf discs. To prevent the mites from escaping the edges of the petri dishes were smeared with Vaseline. Each

experimental petri dish contained 10 immature stages and 10 adult mites. Leaf discs were placed onto filter paper in petri dishes.

Leaf dipping method: Green bean leaf-discs were treated by dipping them into extract solutions of known concentrations, and then allowed to dry for 30 minutes. The treated leaf discs and individual mites were placed in the petri dishes (9 cm in diameter) lined with moistened filter paper. The results were assayed after 1, 3 and 6 days by counting the number of living adults and larvae [17].

Spraying method: Green bean leaf discs were placed into petri dishes on moisturized filter paper. Ten adults were placed in every petri dish. Then adult was sprayed with different concentrations of extract (17-20 $\mu\text{L}/\text{cm}^2$) using a small hand-held sprayer. The results were assayed after 1, 3 and 6 days by counting the number of living adults.

2.4 Ovicidal Effect

Green bean leaf discs were placed into petri dishes on moisturized filter paper and five adult females of the same age were put on the leaf discs and allowed to lay eggs. The eggs were counted after two days. Ten eggs were left in every petri dish and the other eggs removed. Then eggs were sprayed with different concentrations of extract (17-20 $\mu\text{L}/\text{cm}^2$) using a small hand held sprayer. The numbers of hatched larvae were counted. The experiments and a control were replicated 10 times.

2.5 Effect on Egg Laying Capacity

Green bean leaf discs dipped for 3-5 seconds in prepared concentrations (1%, 3%, 6%, 12%) were then dried for 30 minutes and placed in petri dishes with ten adults. After 48 hours of feeding on treated green bean leaves, mites were given untreated green bean leaves. The experiment was repeated 10 times. Daily monitoring was done for fourteen days and the total number of eggs was recorded [18].

All of experiments were conducted in a climate

chamber at 25 ± 1 °C, relative humidity $65 \pm 5\%$ and under long daylight (16L:8D).

2.6 Statistical Analysis

Mortality data were analyzed using a binary logistic regression model allowing for control mortality as outlined by Abbott [19]. The obtained results were submitted to a variance analysis and the mean values were compared by Duncan's test. Significant differences ($p < 0.05$) were calculated and LD₅₀ values were determined by probit analysis (SPSS 20.6). Egg numbers were analyzed using a Poisson regression model with concentration as a predictor. Both extracts were assumed to have a common intercept, but different slopes. All modeling was carried out using SAS version 9.4.

3. Results and Discussion

3.1 Ovicidal Effect

All of the treated eggs were hatched, therefore it is revealed that the ethanolic extracts of the *H. niger* and *H. calycinum* did not have any ovicidal effect and the larvae developed normally (Table 1).

3.2 Acaricidal Effect on Immature Stages and Adults

Leaf dipping data are shown in Table 2. The highest mortality rate was at concentration. The highest effect occurred at concentration of 12% while the smallest effect was for 1%. Statistical analysis showed $p < 0.05$ importance between the treatments ($p < 0.05$), but there was no difference between concentrations 6% and 12%. For the adults placed on leaf discs treated

with *H. niger* extract, the highest effect occurred the 12% concentration. For *H. calycinum*, the larval mortality on leaf discs treated with concentrations was as high as 83%. The highest effect was related to concentration. For the adults placed on *H. calycinum* treated leaf discs, the highest effect occurred at 12% concentration. There were significant differences ($p < 0.05$) between the treatments, but there was no difference between concentrations 6% and 12%.

For the *H. niger* leaf spraying method, highest larval mortality occurred at 12% concentration. Increased concentration leads to increased larval mortality. Statistical analysis showed significant differences between the treatments (Table 2). For adults sprayed with different concentrations of extract, the highest occurred at the 12% concentration. Statistically significant differences were found between the treatments ($p < 0.05$), but there was no difference between concentrations 6 and 12%.

For the larvae placed on *H. calycinum* treated leaf discs, the highest mortality occurred at the 12% concentration. Increased concentration led to increased larval mortality. Statistical analysis showed significant differences between the treatments (Table 2). For adults sprayed with different concentrations of *H. calycinum* extract, the highest effect occurred at the 12% concentration. Statistical analysis showed significant differences among the treatments, but there was no difference between concentrations 1%, 3% and 6%. There was not statistically significant difference between leaf dipping and direct spray.

Table 1 The ovicidal effects of extracts obtained from two different plants on the eggs of *Tetranychus urticae* (Mean $\pm$ SE).

Concentrations (%)	Treatments	
	<i>H. niger</i>	<i>H. calycinum</i>
	Number of hatched eggs	
Control	98	97
1	96	94
3	95	97
6	97	96
12	96	97

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Table 2 The effects of extracts obtained from two different plants on *Tetranychus urticae* (Mean $\pm$ SE).

Treatment	C (%)	Leaf dipping method				Leaf spraying method			
		Larvae		Adult		Larvae		Adult	
		M. (%)	Effect (%)	M. (%)	Effect (%)	M. (%)	Effect (%)	M. (%)	Effect (%)
<i>H. niger</i>	1	50	35.52 $\pm$ 4.23c*	36	29.77 $\pm$ 4.67c	67	59.53 $\pm$ 2.18c	59	51.42 $\pm$ 3.32c
	3	71	62.86 $\pm$ 3.27b	70	63.74 $\pm$ 3.98b	80	73.94 $\pm$ 3.81b	73	68.37 $\pm$ 2.78b
	6	84	79.52 $\pm$ 2.00a	85	82.49 $\pm$ 3.95a	92	91.19 $\pm$ 2.55a	86	83.77 $\pm$ 2.47a
	12	90	87.13 $\pm$ 0.32a	92	81.66 $\pm$ 4.20a	95	93.38 $\pm$ 2.22a	91	89.58 $\pm$ 3.21a
<i>H. calycinum</i>	1	51	42.31 $\pm$ 2.42d	55	46.94 $\pm$ 2.03c	53	40.29 $\pm$ 2.67d	65	58.79 $\pm$ 1.96b
	3	75	55.18 $\pm$ 1.58c	72	63.38 $\pm$ 2.31b	62	51.81 $\pm$ 1.41c	68	62.25 $\pm$ 2.41b
	6	77	70.73 $\pm$ 2.35b	78	75.27 $\pm$ 1.05a	72	63.25 $\pm$ 2.70b	72	67.24 $\pm$ 4.22b
	12	83	77.77 $\pm$ 2.28a	84	80.97 $\pm$ 2.66a	80	74.62 $\pm$ 3.11a	87	84.59 $\pm$ 2.53b
Control		22	0	15	0	22	0	21	0

* Means within rows followed by the same letter are not significantly different ($p < 0.05$, Duncan's multiple range test).

M.: Mortality C.: Concentrations.

Table 3 LC₅₀ values of two different plants extracts against *Tetranychus urticae* using leaf dipping and leaf spraying bioassay.

Treatment	Leaf dipping method				Leaf spraying method			
	Toxicity regression line	LC ₅₀ (w/v)	95% confidence interval	χ^2	Toxicity regression line	LC ₅₀ (w/v)	95% confidence interval	χ^2
Larva								
<i>H. niger</i>	0.31x – 8.26	5.47	4.32 – 6.94	24.65	0.11x – 13.45	3.32	2.31 – 4.77	28.89
<i>H. calycinum</i>	0.24x – 9.23	9.62	6.05 – 15.29	31.83	0.24x – 7.25	12.88	8.64 – 19.20	16.04
Adult								
<i>H. niger</i>	0.24x – 7.25	1.17	1.69 – 2.69	16.47	0.07x – 12.94	0.56	0.64 – 1.61	21.57
<i>H. calycinum</i>	0.08x – 7.22	2.13	0.64 – 2.12	14.77	0.25x – 7.19	1.02	0.12 – 2.64	23.82

The values of LC₅₀ determined are given in Table 3. As shown the table, for dipping methods, LC₅₀ values of crude extracts *H. niger* were determined lower than extracts of *H. calycinum* both larva and adult stage. In spraying methods, the results were observed similar to dipping methods extracts of *H. niger* and *H. calycinum*.

3.3 Effect on Reproductive Capacity

The number of eggs laid by mites feeding on extract-treated bean leaves was significantly ($p < 0.05$) related to concentration for both extracts. The fitted Poisson regression model is:

$$\text{Number eggs} = E_0 e^{-kC}$$

where, E_0 is the number of eggs laid under control conditions, C is the extract concentration and k is an estimated rate. Initially we fitted a common intercept

but differing rates of decline for each extract. However, tests showed no significant difference between the two curves ($p = 0.67$), so the final fitted model ignored extract. The fitted model was $Eggs = 183.1e^{-0.1515C}$. From this, we can estimate that the number of eggs laid halves for each 4.6-unit increase in concentration (Fig. 1).

Ethanolic extracts were obtained from *H. niger* and *H. calycinum* and their effect was first tested on *T. urticae*. It was observed that the extracts showed a high rate of larvae mortality of adult and reduced fecundity on the female of *T. urticae*. Application of plant extracts to control harmful microorganisms has been used for hundreds of years. For the past decades, acaricidal and insecticidal properties of plant extracts have been widely used against harmful pests. Previous studies revealed that the three commercial preparations

Fig. 1 The effects on reproductive capacity of extracts obtained from two different plants on *Tetranychus urticae* (Mean $\pm$ SE).

of neem seed extracts (Margosan-0 and Neem Azal S, Neem Azal T/S) were effective on *T. urticae* [17, 21]. In addition, several herbal extracts of *Achillea millefolium*, *Taraxacum officinale*, *Matricaria chamomilla* and *Salvia officinalis* showed strong inhibition of the feeding activity of mites [22-24]. It was found that the extracts of yew a high mortality of adult, decrease in female fecundity and shortened longevity [25, 26]. Shi et al. [27] revealed that the extract of *Chenopodium scoparium* showed contact and systemic effects and caused high rates of mortality in all three species (*T. urticae*, *T. cinnabarinus*, *T. viennensis*). It was determined that the extract of *Kochia scoparia* had the highest mortality on mites (*T. urticae*, *T. cinnabarinus*, *T. viennensis*) [28]. The extracts of wild tomato leaf showed strong repellency effect on two-spotted spider mite [28]. Wei et al. [29] found that the extracts of *Aleo vera* L. showed acaricidal effect with LC_{50} value of 90 ppm on *T. cinnabarinus*. In addition, it was revealed that the leaf

extracts of *Juglans regia* had strong effect on *T. cinnabarinus*, *T. viennensis* and the value of LC_{50} of the extracts from petroleum ether, chloroform, ethyl acetate, methanol, and distilled water to mite species were 0.73, 1.66, 4.96, 7.45 and 9.91 mg/mL respectively [30]. Crude methanolic extraction of foliar extraction of 67 plant species belonging to subfamilies of Australian Lamiaceae showed two different effects as contact and systemic toxicity to the two spotted mites [31]. According to the results presented in Table 1, the mortality of *T. urticae* behaved in a concentration-dependent manner. There were no references in the literature to other studies using *H. calycinum* and *H. niger* ethanolic extract against *T. urticae*. There were a few references for the effect of *H. niger* extracts on insects. For example, it was determined that extract of *H. niger* and *H. calycinum* exhibited insecticidal activities against *M. persicae* [32, 15]. In addition, the leaves of *H. niger* have been found to deter mice in buildings [33]. In our

findings, *H. niger* had higher mortality (95% in leaf dipping method and 91% spraying method) than *H. calycinum* extracts (80% in leaf dipping method and 87% spraying method). All parts of *H. niger* contain tropane alkaloids called hyoscyne (scopolamine), hyoscyamine (L-atropine), and atropine (DL-hyoscyamine) [34]. We speculate that these compounds have been responsible for greater toxicity on *T. urticae*. However, there are no references to which compound was acaricidal or to insecticidal effects on insects or mites.

The plant extracts showing acaricidal performance also had other activities which affect the biology of the mites, such as fecundity and repellency. Pure azadirachtin curtailed the egg laying and feeding capacity of *T. urticae* [35]. Neem seed kernel extracts and its formulations have been reported to effect adult repellency and fecundity of mite's adults [36, 38]. Erdogan et al. [11] observed a fecundity reduction in *T. urticae* caused by some plant extracts. Pure azadirachtin reduces the number of eggs laid by *T. urticae* [39]. The results shown in Table 3 indicated that ethanol extracts of *H. niger* and *H. calycinum* caused a reduction in the number of eggs laid per female. This is consistent with the results established by Numa et al. [40] who reported that application of plant extracts may affect the oviposition of *T. urticae* females. It was revealed that the number of eggs laid per female decreases when an increasing concentration of a plant extract was applied [41, 42]. In addition, it was observed that 12% concentration of *Capsicum annum* L. (Solanaceae) extract significantly reduced the reproductive capacity of *T. urticae* females [43].

4. Conclusions

It was determined that the extracts of *H. calycinum* and *H. niger* had a high rate of mortality and reduced fecundity effect on *T. urticae*. The results of this study indicated that the ethanolic extract of *H. calycinum* could be useful against *T. urticae* on vegetable plants

grown in IPM and organic systems of agriculture. Further study is needed to identify the active component of these plant extracts responsible for their acaricidal activities under controlled and field conditions.

Acknowledgments

The authors are grateful to Dr. Numan E. Babaroglu for statistical analysis in Plant Protection Central Research Institute Ankara-Turkey. Special thanks to Bernie Dominiak for reviewed and improved an earlier version of this manuscript (Australia). The authors appreciate the guidance given by Mr. Allan Lisle School of Agriculture and Food Sciences, University of Queensland, Australia, in statistical analyses.

Author Contributions

The initial idea of the study was conceived by Pervin Erdogan and collected plants. Pervin Erdogan and Errol Hassan assisted with the design of experimental protocol. Gulcin Saltan and Betul Sever prepared plants extracts. Pervin Erdogan performed all of experiment. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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