ACARICIDE EFFECTS OF *PHLOMIS PUNGENS* WILLD. VAR. *HIRTA* EXTRACTS ON TWO-SPOTTED SPIDER MITE (TSSM - *TETRANYCUS URTICAE* KOCH) (ARACHNIDA: TETRANYCHIDAE)

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ABSTRACT: Plant extracts are used for biological control of several phytofags, especially of *Tetranycus urticae* (Koch) (Acarina; Tetranycidae) with serious economic damages on agricultural products. These pests create serious damages on vegetables, fruits and ornamental plants in Tokat provicne of Black Sea region. In present study, methanol extracts of *Phlomis pungens* Willd. var. Hirta of Lamiaceae family collected from natural flora of Artova district of Tokat province was used to control two-spotted spider mite (TSSM, *T. urticae*). Extracts doses of 2, 4, 8 and 12% were used in experiments. Mortality rates reached to 98% in 12% dose at the end of 144 hours.

KEY WORDS: *Phlomis pungens, Tetranycus urticae*, acaricidal effect

Tetranychus urticae Koch is nourished with plant extract and thus creates chlorosis and rolls in leaves. It ultimately results in about 40-60% yield losses and significant quality reductions. It also causes spread of various harmful virus diseases (Thomas, 1969). Since *T. urticae* creates damages on agricultural lands through sucking plant extracts, results in serious yield and quality losses unless proper control measures were taken and it has a short development period and high reproduction power, thus a rapid population growth, pesticides are used intensively to control the pests and then it gain resistance against the pesticides in a short time (Shih et al., 1976; Sabelis, 1981; Krips et al., 1998).

It is quite hard to control the pests since they gain resistance against pesticides in short time and already used insecticides have various negative impacts on environment, human health and natural balance and may create damages on off-target organisms and leave residues over them (Zettler & Arthur, 2000; Nas, 2004; Khalfi et al., 2008; Nyamador et al., 2010; Wei et al., 2014). Food safety is under threat because of pesticide residues over agricultural products. Thus, generally natural pests and disease control methods (biological control or plant-originated agents) are used for food safety. Bio-insecticides have already reached to 1% share in insecticide sector and the share will probably rapidly increase in near future (Isman, 1997). There are more than 2000 plant-originated insecticides used for several pests and acarids (Öncüer, 2000).

Just because of negative impacts of chemical control practices, plant extracts have been started to be used as an alternative to insecticides in control of pests and diseases. These extracts are also called as herbal insecticides and they are available in nature. They don't release any toxic substance to nature, do not create pollution in water and soil resources and do not leave any residues over the products (Anonymous, 2015). Then, the natural control practices with plant essential oils and extracts have gained great significant in recent years.

Phlomis pungens Willd. var. *hirta* belongs to *Phlomis* genus of Lamiaceae family and it is a long-lasting dicotyledonous weed. The species has a spread over Africa, Asia and Europe. Most of the species have aromatic and medicinal characteristics. Leaves of some species, including *P. pungens*, are used as herbal tea, stimulant, tonic, diuretic and in treatment of ulcer and hemorrhoid and other species have antioxidant and antimicrobial activities. In Turkey, there are 52 taxa belonging to 6 varieties (Demirci et al., 2008; Zhang & Wan, 2008).

Tasdemir et al. (2004) carried out a study with *Pholomis* sp. and investigated the antimicrobial characteristics of hexane, water and methyl alcohol extracts of plant leaves against *Escherichia coli, Pseudomonas aureginosa, Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans*. Researchers found that plant extracts were especially effective against *Phlomis* (Lamiaceae) species. In another study, ethanol extracts and essential oils of plants and fruits of *Phlomis fruticosa* were also found to have antimicrobial effects (Ristic et al., 2000).

Özkan et al. (2016) carried out a study with *Salvia verticillata* and *Phlomis pungens* plants and indicated that these two plants could be used against various infectious as an alternative to antimicrobials.

Erdoğan et al. (2010a) in a study, investigated the acaricides effect of plant extract of *Capsicum annuum* L. (Solanaceae) against *T. urticae*. The researchers used two different methods (dipping and spraying) to determine the acaricides effect of the extracts. Bean leaf discs (3 cm in diameter) were used as nutrient. Four different extract concentrations (1, 3, 6, 12%) were applied and the greatest mortality rates in larva, nymph and mature periods were observed in 12% concentration. The mortality rates in larva, nymph and mature periods in this concentration were respectively observed as 97, 86 and 95%. Significant differences were not observed in mortality rates of dipping and spray methods. In another study carried out by Erdoğan et al. (2010b), common marrow extracts resulted in significant mortality rates in larva, nymph and mature stages of *T. urticae*.

MATERIAL AND METHODS

Material

The extracts of *Phlomis pungens* Willd. var. *hirta* Velen plants collected from Artova district of Tokat province and *Tetranychus urticae* Koch individuals constituted the primary material of this study.

Method

Phlomis pungens Willd. var. *hirta* Velen

Because of phenological period, the *Phlomis pungens W*illd. var. *hirta* Velen plants used in this study were collected from Artova (Tokat) district between June-August. Plant diagnosis was carried out by Asst. Prof. Dr. Bedrettin Selvi at Biology Department of Gaziosmanpaşa University Faculty of Science.

Tetranychus urticae Koch cuture

The *Tetranychus urticae* Koch (Acarina: Tetranychidae) used in this study were grown in stock culture over bean plants in a production cabin at Plant Protection Department of Gaziosmanpaşa University Agricultural Faculty. Plants were kept as fresh and population was sustained through contaminating to healthy plants.

Collection and drying of *Phlomis pungens* Willd. var. *hirta* Velen plants

The *Phlomis pungens* Willd. var. *hirta* Velen plants were collected at flowering periods. Samples were taken from full plants and a herbarium was formed. Coordinates of collection locations were recorded with a GPS device and plants were kept for drying under non-humid room conditions at 25±1 °C temperature.

Ethanol extracts

To get plant extracts, 100 g ground plant material was weighed, placed into 1000 ml Erlenmeyer flasks and supplemented with 600 ml ethanol. Samples were then covered with para-film and shaken at orbital shaker for 24 hours. They were filtered through drying papers (Dabrowski & Urszula, 2007) and extracts were obtained through evaporating ethanol in a Rotary evaporator. Resultant extracts were kept at +4 °C.

Ethanol extract treatments

The extract used in this study was dissolved in acetone and dissolved extract was supplemented with %3 Tween 20 drops. Then, single dose treatments were performed to determine the application doses. Beside effective dose, 4 different doses were also determined after this single dose. The non-infected bean leaves were cut in 3 cm diameter and dipped into extract solution for 5 s. Following the dipping duration, leaves were left for drying for 30 minutes, they were placed in petri dishes with moist cotton inside and 12 adult mites were placed over the leaves. There were 3 control treatments as of acetone-Tween, distilled water-Tween and distilled water. Mortality counts were performed under microscope for 10 days and acaricide effect was looked for with these treatments. Experiments including 4 different doses (2, 4, 8 and 12%) were conducted in randomized blocks experimental design with 3 replications.

Statistical analyses

Experimental data were subjected to statistical analyses with SPSS software and effects were calculated in accordance with Abbott (1925). Values were calculated as percentages. Data were subjected to analysis of variance (ANOVA) ($P \leq 0.05$) and differences between treatments were tested with Tukey multiple range test ($P \leq 0.05$).

RESULTS

Ethanol extracts were applied in 4 different doses in present experiments and mortality rates in leaves treated with dipping method were determined (Table 1).

There were significant differences in mortality rates of two-spotted spider mite (TSSM) under different doses of plant extract. Variance analysis revealed a relationship between the time and the dose and effects were observed from the second hour. While the mortality rate was 8% at the end of 24 hours of the least dose (2%), the rate reached to 56% at the end of 120 hours. At a higher dose (8%), the mortality was 35% at the end of 24 hours and 83% at the end of 120 hours. The mortality rate at the greatest dose (12%) was recorded as 42% at the end of 24 hours, 75% at the end of 72 hours and 90% at the end of 120 hours (Figsç 1-4).

DISCUSSION

Contact and systemic effects of *Chenapodium scoparium* on *Tetranychus urticae, Tetranychus cinnabarinus* and *Tetranychus viennensis* species were reported (Shi et al., 2006). In a previous study carried out with 67 plant extracts of Lamiaceae family, Rasikari et al., (2005) reported contact effect of plant

extracts on *T. urticae*. Contact and systemic effects of extract of *Juglans* sp. leaves on *T. cinnabarinus* and *T. viennensis* were also reported by Wang et al. (2007).

Biological control of spider mite as an alternative to pesticides is also an expensive process and there are specific predator acarides like *Phytoseilus persimilis* and *Amblyseius californicus* (Cherian, 2003; Labuchagne, 2005). However, plant-originated preparates are still advantageous over the chemical pesticides.

CONCLUSION

In present experiments, a mortality rate over 50-60 % was achieved with in-vitro *P. punges* treatments at the end of 120 hours. Such a rate reached to 100% at the end of 216 hours. It was concluded that such treatments may also be effective for biological control of spider mite under field conditions.

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Table 1. Min-max mortality rates (%) of *Tetranychus urticae* Koch mature individuals treated with different doses of *Pholomis pungens* plant extract (mean±SD).

	Sayım zamanı (saat)					
Dozlar (%)	2. saat	24. saath	48. saat	72. saat	96.saat	120. saat
Kontrol	0.00±0.00a	0.00±0.00a	4,16±2,08a	8,33±2,08a	10,41±2,08a	16,66±2,08a
2	0.00±0.00a	7,63±2,16a	19,44±2,58a	29,16±2,40b	40,97±3,31b	55,55±2,58b
4	0.69±0.69a	10,41±2,08a	20,83±2,61a	35,41±2,73b	56,25±3,42bc	68,05±4,45bc
8	22,91±5,43ab	37,50±5,17b	20,83±2,61a	73,61±4,56c	79,16±4,52cd	84,72±3,81cd
12	9,72±2,87b	42,36±2,80b	43,75±5,99b	65,27±2,25c	72,91±2,08d	89,58±2,08d

P<0.05** The means in the same column indicated with different letters are significantly different.

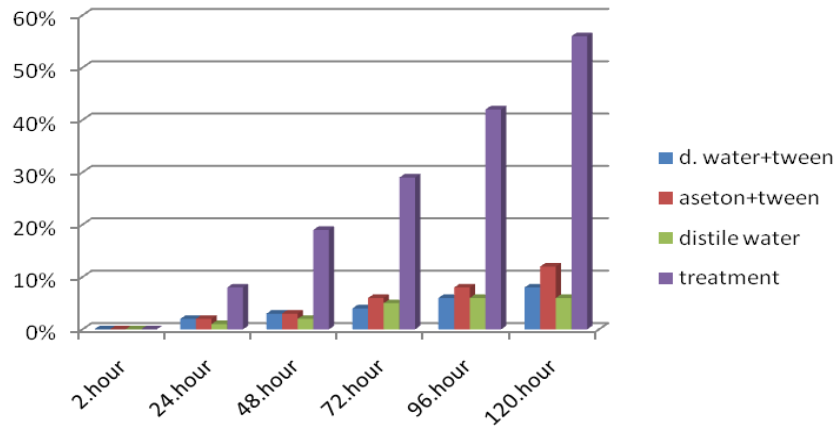

Figure 1. Mortality rate 2%

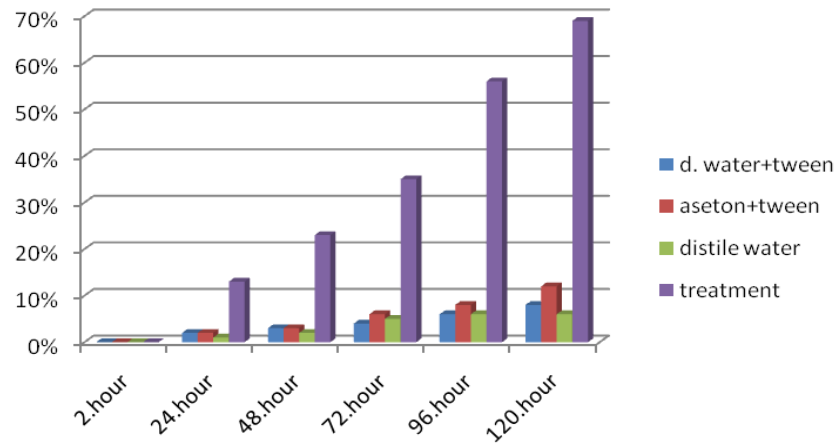

Figure 2. Mortality rate 4%.

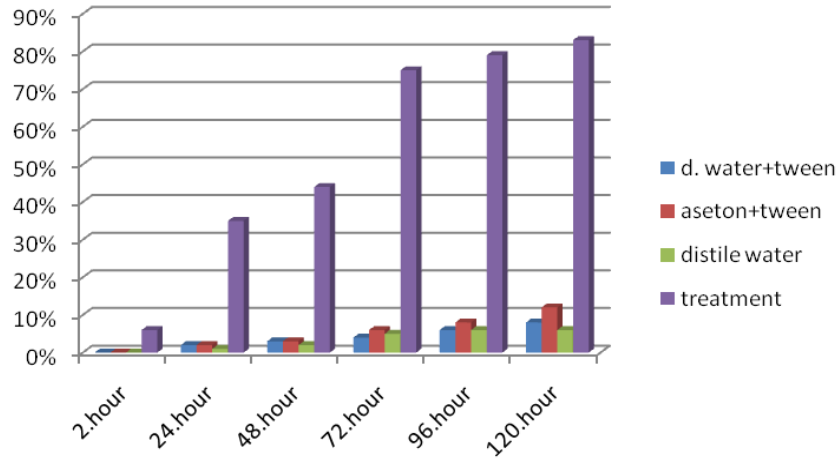

Figure 3. Mortality rate 8%.

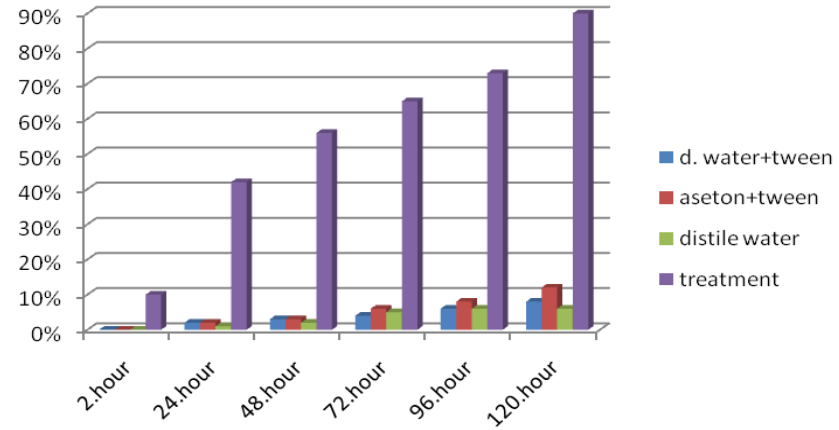

Figure 4. Mortalty rate 12%.