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INSECTICIDAL EFFECT OF EXTRACTS FROM SIX NATIVE PLANTS ON *Bemisia tabaci* AND SOME PHYSIOLOGICAL EFFECTS ON CUCUMBER AS HOST PLANT

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In this study, the effect of six endemic plants' extracts (i.e. *Satureja sahendica*, *S. khuzistanica*, *Scrophularia striata*, *Thymbra spicata*, *Oliveria decumbens* and *Vitex agnus castus*) as well as commercial insecticide (Acetamiprid: Mospilan®) was investigated against *Bemisia tabaci* (Hemiptera: Aleyrodidae) and some physiological responses in cucumber as host plants. The plant extracts and Acetamiprid as chemical pesticide were sprayed on the plants with 250, 500 and 1000 ppm concentrations. All the extracts exhibited significantly different mortality of adults as compared with the control. The extracts of *O. decumbens* showed the high mortality percentage of adult population as compared with other treatments. Results showed that the application of extracts and pesticide, especially in high concentrations, showed negative effects on plant and significantly increased malondialdehyde (MDA) accumulation and chlorophyll, proline and carbohydrate accumulation in cucumber leaves. Moreover, foliar spray with low concentrations of extract (250 and 500 ppm) enhanced cholorophyll content and decreased MDA and proline accumulation compared with control and Acetamiprid treated plants. It was concluded that these six extracts could be used as effective and environmentally sustainable bio-insecticides for the control of *B. tabaci* (B biotype) without any adverse effects on host plants.
Keywords: Aromatic, chlorophyll, essential oils, Lamiaceae, pesticide, proline.

INTRODUCTION

The cucumber (*Cucumis sativus* L.) belongs to family Cucurbitaceae and one of the important cultivated vegetable in greenhouse and open-field systems in Iran and other countries. Iran had about 66,146 ha cucumber production area and produced 1.57 million tons in 2013 (FAO, 2013). There are several main pests and diseases affecting cucumber in Iran, amongst them, *Bemisia tabaci* is an important pest which has caused high losses in yield with direct feeding and transmission of plant viruses (Oliveira *et al*., 2001; Morales, 2007).

At present, synthetic chemicals are used for control of whitefly. However, applications of the synthetic insecticides have not been totally effective, partially owing to the waxy cover of the pest. This waxy cover prevents penetration of insecticides and deters contact with the pest's sedentary nymphal and pupal stages (James, 2003). In addition, the application of synthetic pesticides may cause health hazards for user and environment and residual accumulation happens on the consumer (Lamiri *et al*., 2001). Therefore, the harmful effects and high cost–benefit ratio of chemical pesticides have motivated the researchers to develop alternatives to chemical control in pest management. Medicinal and aromatic plants extracts may provide an alternative to the currently used pesticides for the control of plant pests, as they constitute a rich source of bioactive chemicals (Daoubi *et al*., 2005). Pesticides-derived from aromatic plants are more eco-friendly than synthetic chemicals because of their low mammalian toxicity, reduced effect on non-target organisms and low biodegradability in the environment (Georges *et al*., 2008). Recently, some studies have been done on the plants extracts and essential oils against to weeds, pests and phytophagous insects (Aslan *et al*., 2004; Batish *et al*., 2008; Yang *et al*., 2010; Ameri *et al*., 2011). The main objectives of this study were to evaluate the effects of plant-derived extracts on *Bemisia tabaci* and some physiological responses in cucumber as host plant.

MATERIALS AND METHODS

***Plant material and host plant growing conditions*:** This study was conducted in cucumber fields of Darre Shahr, Ilam province (west of Iran) in the year 2014, through a randomized complete block design (RCBD) with three replications. Seeds of cucumber cv. 'Vilmorin', were cultured with a spacing of 0.5×0.5 m. The buffer zone between two experimental units was 0.5m, while the distance from one replication to the other was 1m. All cultural practices i.e., irrigation, hoeing and weeding were carried out throughout the growing season as recommended.

***Plant extraction*:** Six medicinal plants were used in this study i.e., *Satureja sahendica* (Lamiaceae), *Satureja khuzistanica* (Lamiaceae), *Scrophularia striata* (Scrophulariaceae), *Thymbra spicata* (Lamiaceae), *Oliveria decumbens* (Apiaceae) and *Vitex agnus castus* (Verbenaceae). Aerial herbage of the plants was collected in their flowering stage from natural habitat of Ilam province. Harvested shoots were air dried at shade for 10 days before the dried parts were grounded. Then, 50 grams of plant powder was transferred to dark-colored flasks on shaker and mixed with 400 ml of ethanol stored at room temperature. After 30 min, infusions were filtered and residues were re-extracted with 300 ml of ethanol as solvent for two times, and finally were stored in a refrigerator at 4°C.

***Treatment imposition*:** Whitefly adults were collected on cucumber fields. To obtain uniform age insects for experiment, vigorous whitefly adults (*Bemisia tabaci*, B biotype) were selected from the population to produce eggs on insect free cucumber plants for 36 h. The adults were removed to allow eggs to uniform age to development in a laboratory chamber at 24±1°C, 70±5% RH and 16:8 h light:dark photoperiod for a certain period to obtain vigorous two-day-old adults (Wang et al., 2007). These adults were used in all the experiments. Whitefly cultures were established by transferring 100 unsexed adults onto cucumber plant in insect-proof cages in a field condition.

Plant extracts were evaluated against *B. tabaci* populations using cucumber as the host plant. Three concentrations (i.e. 250, 500, and 1000 ppm) of the extracts and Acetamiprid (Mospilan®) pesticide as positive chemical control were applied over the adaxial and abaxial surfaces of the leaves. In untreated control, plants were sprayed with distilled water. After 48 hours of foliar spray, trials were measured for the following reasons: (1) effect of extracts on *B. tabaci* adults: for each experiment, three leaves of cucumber plant were detached and the number of adult whiteflies was recorded. The decrease in percentage of the adult population in each treatment was calculated based on the number of killed whitefly adults/number of introduced whitefly adults × 100; (2) Chlorophyll contents determination were calculated using the equations proposed by Strain and Svec (1966); (3) Proline content was determined according to the method described by Bates *et al*. (1973) with some modification; (4) the Malondialdehyde (MDA) content was calculated from the subtracted absorbance (A532–A600) using the extinction coefficient of 155 m m^{-1} cm^{-1} (Stewart and Bewley, 1980); (5) and Carbohydrate content was measured based on the Anthrone method (Irigoyen *et al*., 1992).

***Statistical analysis*:** Statistical analysis was performed using SAS (version 9.1) software (SAS, 2002), and ANOVA were performed, and the means were compared using the Duncan's Multiple Range Test at p=0.05.

RESULTS

***Mortality percent of adults*:** The insecticidal effects of six medicinal plant extracts and Acetamiprid as synthetic pesticide against whiteflies were shown in Figure 1. The results indicated that all six extracts have toxic effects on whitefly as compared with control. Results showed that extracts of all six plants, significantly reduced population of *B. tabaci* on cucumber leaves. Among the six studied plants, the extracts of *Oliveria decumbens* in 1000 ppm concentrations exhibited the strongest toxicity against adults of whitefly compared to others and chemical control, followed by the extract of *Scrophulari astriata* and *Satureja khuzistanica*.

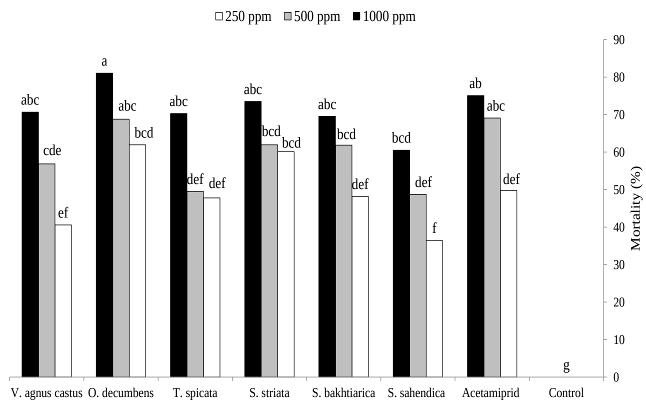

Figure 1. Effect of plants extracts and Acetamiprid on percent mortality of *B. tabaci.*

***Total chlorophyll content*:** Leaves of cucumber plants sprayed with plants' extracts and Acetamiprid, especially in 1000 ppm concentrations, showed a significant reduction in chlorophyll content compared with control plants (Fig. 2). Indeed, after treatment with 250 and 500 and 1000 ppm extract of *Oliveria decumbens* chlorophyll content was maintained compared with other extracts. The highest reduction in chlorophyll content was observed in 1000 ppm of Acetamiprid, *S. khuzistanica, T. spicata* and *V. agnus-castus*. Results revealed that in all treatments total chlorophyll accumulation decreased in cucumber leaves as the concentration of extracts increased.

***Malondialdehyde content*:** The effects of different treatments on MDA accumulation in cucumber leaves are summarized in Figure 3. Results indicated that foliar application of extracts and Acetamiprid significantly increased MDA accumulation in cucumber plants, especially in those treated with 1000 ppm concentrations. Meanwhile, the lowest MDA content was obtained in plants treated with 250 ppm of *O. decumbens* extract, followed by 250 ppm of *S. striata* extract. Cucumber leaves MDA accumulation, increased as the doses of treatments increased. In addition, results indicated that all six extracts in 250 ppm concentrations significantly decreased MDA accumulation in leaves as compared with control and Acetamiprid application.

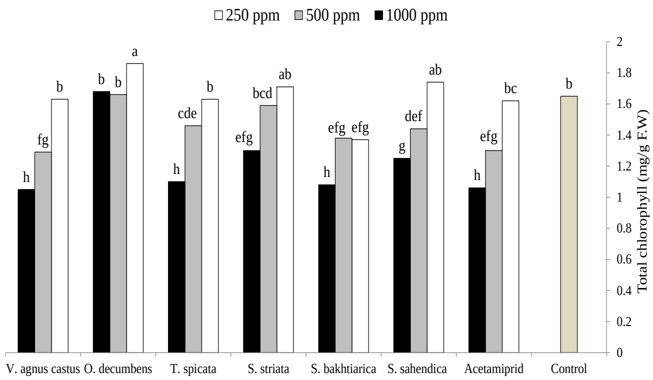

Figure 2. **Effect of plants extracts and Acetamiprid on total chlorophyll of cucumber plants.**

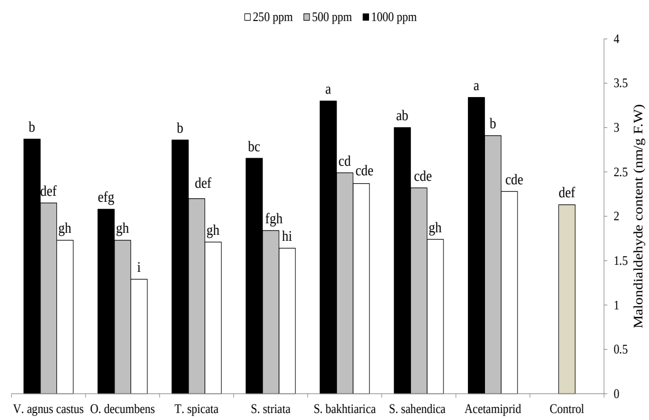

Figure 3. **Effect of plants extracts and Acetamiprid on total Malondialdehyde content of cucumber plants.**

***Proline and carbohydrate content*:** Results showed that proline and carbohydrate content in leaves significantly increased in plants exposed to 500 and 1000 ppm of plants extracts and 250, 500 and 1000 ppm of Acetamiprid as compared with control plants (Figures 4 and 5). In addition, the lowest content was obtained in plants treated with 250 and 500ppm of *O. decumbens* extract, followed by 250 ppm of *V. agnus-castus* and control plants. The increase of proline and carbohydrate amount was extracts-dosedependent so that the higher extracts dose, the more accumulation of proline in leaves. The highest proline content observed in plants sprayed with Acetamiprid and *S. sahandica* in 1000ppm concentrations.

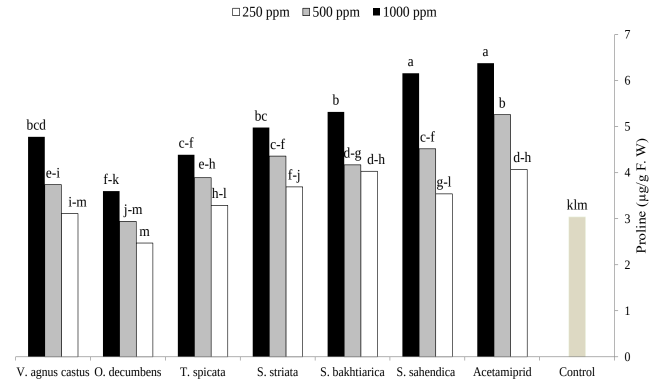

Figure 4. Effect of plants extracts and Acetamiprid on Proline content of cucumber plants.

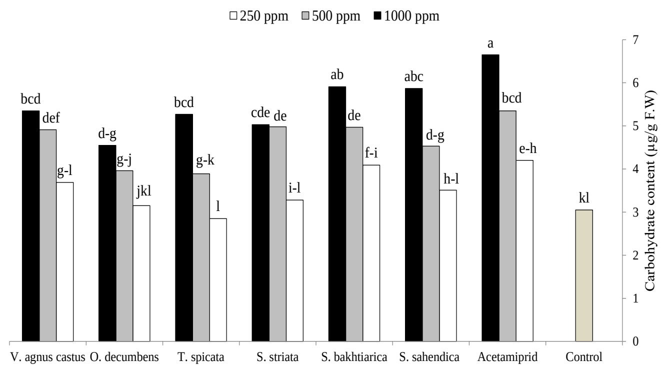

Figure 5. **Effect of plants extracts and Acetamiprid on carbohydrate content of cucumber plants.**

DISCUSSION

The present study revealed that extracts obtained from six plans species sprayed on cucumber, efficiently killed adults whitefly. *Oliveria decumbens* and *Scrophularia striata* extracts at concentration of 250, 500 and 1000 ppm had more efficiently on whiteflies population as compared with Acetamiprid and control. In this relation, the essential oils obtained from *Satureja hortensis*, *Thymus vulgaris* and *Ocimum basilicum* were reported as efficient against the adults of whitefly and two-spotted spider mite, *Tetranychus urticae* (Aslan *et al*., 2004). Yadav and Mendhulkar (2015) also showed that the aqueous extract of *Couroupita guianensis* had moderate pesticide activity against *B. tabaci*. There is no report on pesticide activity or chemical constituents of the indigenous species viz., *Satureja sahendica*, *Satureja khuzistanica*, *Scrophularia striata*, *Thymbra spicata*, *Oliveria decumbens*, and *Vitex agnus castus*, was previously available. However, the toxic effects of aromatic plants extracts and essential oils against insect pests are reported in some other publications (Batish *et al*., 2008; Yang *et al*., 2010). The insecticide activity of aromatic plants' extracts against pests can be attributed to the essential oil components (Isman, 2006). The previous studies have shown that the toxic effects of extracts and essential oils from aromatic plants against insect pests are related to their main components (Cruz-Estrada *et al*., 2013; Zoubiri *et al*., 2014). Among these six medicinal plants, the extract from *O. decumbens* was the most effective. Better toxicity effect of the aromatic plant may be explained by higher thymol (47.06%) and Carvacrol content (23.31%) (Amin *et al*., 2005). The extracts obtained from six plants showed good results as insecticidal efficacy, especially *Oliveria decumbens* and *Scrophularia striata* to be used in sustainable pest management in the greenhouses and open fields, but need further studies.

Natural herbal products may affect other plants in their vicinity in a stimulatory or inhibitory manner such as allelopathy (Prasanta *et al*., 2003). Therefore, the studies of allelopathic interactions between plants are one of the most important strategies for herbicide discovery (Grichi *et al*., 2016).

Results of the present study showed that application of 500 and 1000 ppm of herbal extracts and Acetamiprid as a chemical pesticide, with the exception of *O. decumbens* extracts decreased chlorophyll content in cucumber leaves in comparison to control plants. The observed loss in chlorophyll content is in agreement to earlier studies reporting that essential oils reduce chlorophyll content and thus interferes with photosynthetic activity of the plants (Batish *et al*., 2004). The decrease in leaf chlorophyll content due to allelopathic effects of essential oils has been also reported. The allelochemicals can stimulate the degrading pathway of chlorophyll and decrease its accumulation which in turn affects photosynthesis process and diminishes the total plant growth (Peng *et al*., 2004; Siddiqui and Zaman, 2005). In addition, the reduction effect of volatile oils from *Artemisia scoparia* on chlorophyll content and cellular respiration in weeds was demonstrated (Kaur *et al*., 2010). Masuda *et al.* (2016) reported that infestation by the sweet potato whitefly inhibits the accumulation of carotenoids and chlorophyll in tomato and squash plants which are both synthesized through the non-mevalonate pathway. In this experiment, the enhancement of chlorophyll content in *O. decumbens* extracts-treated plants under whitefly contamination was observed. These results suggested that cell structure of cucumber leaves under *B. tabaci* contamination received less damage after pretreatment with *O. decumbens* extracts.

Lipid peroxidation level in leaves of cucumber plants was assessed by MDA content. Results demonstrated that spraying cucumber plants with 500 and 1000ppm of six extracts and Acetamiprid is resulted in increasing MDA content in comparison to control plants. The oxidative degradation of lipids induced lipid peroxidation and enhanced MDA accumulation is an indicator of cell wall lipids peroxidation (Heath and Packer, 1968). Some experiments have showed that essential oils from various aromatic plants caused accumulation of MDA in some plant species (Yu *et al*., 2003; Grichi *et al*., 2016). The amount of MDA content can be served as benchmark for determining the extent of damage to plants due to biotic and abiotic stresses. In this experiment, the rate of MDA was increased as the herbal extracts concentration increased. Application of some extracts such as *O. decumbens*, *S. striata*, *S. spicata*, *S. sahandica* and *V. agnus-castus* in low concentration significantly reduced the MDA content in cucumber leaves in comparison to Acetamiprid treated and control plants. It is believed that their effects on the stability of cellular membrane caused the reduction of MDA on plants under the whitefly contamination.

Plants have developed several defense mechanisms including compatible solutes such as proline and carbohydrate accumulation to prevent the cellular damage due to reactive oxygen species (ROS) generation in biotic and abiotic conditions (Singh *et al*., 2006). Our results indicated that extracts of six aromatic plants and Acetamiprid as a chemical control, with the exception of *O. decumbens* induced a significant accumulation of proline and carbohydrate in the leaf tissues of cucumber plants. Some studies have reported that volatile oils from various allelopathic plants and their constituents caused accumulation of proline and carbohydrate in some plant weeds and species (Ameri *et al*., 2011). The reasons of accumulation of the amino acid in cells are numerous and various. Some factors such as preventing decomposing proline, preventing from converting proline to protein or increasing the decomposing of proteins have been reported as the reasons for accumulation of proline during experiencing stresses. The rising proline accumulation in plants while exposing to environmental stresses is a kind of defense mechanism by which the activities of enzymes forming hydroxyl radicals will be blocked and the capacity of plants for tolerating environmental stresses will be improved (Mohammad and Akladious, 2014). In this study, application of plants extracts in low concentrations (250 ppm) significantly led to maintain the content of proline and carbohydrate in comparison with control plants and reduced proline and carbohydrate content in comparison to plants treated by Acetamiprid. The increase of proline content was concentration-dependent. These results revealed that leaf tissues of cucumber plants under *B. tabaci* contamination received less damage after spraying with extracts in comparison to chemical pesticide.

***Conclusion*: ** In conclusion, the present founding indicate that despite the chlorophyll reduction and accumulation of MDA and proline observed in application of high concentrations probably due to oxidative stress, extracts from all six plants, especially *Oliveria decombens* at low concentrations (250ppm) were extremely efficient in controlling of adult population of *B. tabaci* without adverse effects on host plants compared with the applied commercial insecticide.

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