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THE EFFECT OF SOME HERBAL EXTRACTS AND PESTICIDES ON THE BIOLOGICAL PARAMETERS OF *BEMISIA TABACI* (GENN.) (HEM.: ALEYRODIDAE) PERTAINING TO TOMATO GROWN UNDER CONTROLLED CONDITIONS

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Abstract: The sweet potato whitefly, *Bemisia tabaci* (Genn.) (Biotype A: Hem.: Aleyrodidae) is one of the most important pests in Iran. The development of alternative methods that are not chemical applications, is necessary in pest management for the sake of human health and for the safety of the environment. In this research project, the effect of four herbal compounds *Fumaria parviflora* Lam. (Fumariaceae), *Teucrium polium* L. (Lamiaceae), *Calotropis procera* (Willd.) R. Br. (Asclepiadaceae), *Thymus vulgaris* L. (Lamiaceae) on the same biological parameters of cotton whitefly were compared with the insecticides azadirachtin and pymetrozin.

The results showed significant differences between herbal compounds and insecticides on: egg longevity ($F_{6, 39} = 2.96$, $p < 0.05$), pupa longevity ($F_{6, 37} = 4.49$, $p < 0.01$) and adult longevity ($F_{6, 31} = 4.47$, $p < 0.01$); egg mortality ($F_{6, 39} = 6.71$, $p < 0.001$), total egg mortality ($F_{6, 39} = 6.71$, $p < 0.001$), sex ratio (female) ($F_{6, 30} = 3.49$, $p < 0.01$), oviposition period ($F_{6,31} = 7.50$, $p < 0.001$) and total fecundity/female ($F_{6, 31} = 8.23$, $p < 0.001$).

These results indicated that a fumitory extract and pymetrozin had the best effect on longevity and mortality, respectively. According to the results, the fumitory had a noticeable effect on the different life stages of the sweet potato whitefly.

Key words: *Bemisia tabaci*, cotton whitefly, tomato, biological parameters and herbal compound

INTRODUCTION

Cotton whitefly, *Bemisia tabaci* Gennadius biotype A, (Hemiptera: Aleyrodidae) is one of the most destructive insect pests of agricultural, horticultural, ornamental crops and greenhouse crops, especially tomato (Jones 2003). Tomato is grown in both tropical and subtropical regions, under protected cultivation (Butler *et al*. 1986; Denholm *et al*. 1996). This species is considered destructive due to a number of factors: high degree of polyphagy, ingestion of phloem sap, massive honey dew secretion that reduces both the cosmetic value of the tomato and the available leaf area for photosynthetic activities, uneven ripening in tomatoes, and transmission of plant viruses (Duffus 1987; Maynard and Cantliffe 1989; Byrne *et al*. 1990; Rapisarda and Garzia 2002). Chemical control methods cannot control the population of this pest because of its nutrition patterns, oviposition, and the mating and growth of nymphs that occur under leaf surfaces (Coudriet *et al.* 1985). Pest control is a main part of an agricultural economy. In modern approaches, using new pesticides is an essential factor for increasing safe production and protecting the environment. Unfortunately, repeated spraying of dangerous poisons is the most important factor in pest resistance (Gerling 1990).

Development of resistance to existing conventional pesticides, the increasing environmental pollution and health dangers created by synthetic pesticides mean that there is a great need for new kinds of useful pest control agents with higher activity against the target pests, and a lower impact on humans and the environment (Sayeda *et al*. 2009). Plants may provide an alternative to the currently used insecticides for the control of insect pests. Plants constitute a rich source of bioactive chemicals (Kim *et al*. 2005). Monoterpenes, sesquiterpene lactones, and triterpenes are examples of such metabolites that may have commercial usage (Heywood *et al*. 1977; Barney *et al*. 2005). Medicinal plants not only sooth human sufferings but also are used as insecticides (Koschier and Sedy 2003). The use of herbal compounds or plant pesticides goes back a few hundred years in China, Egypt, Greece and ancient India (Pascual-villalobos and Robledo 1998).

Plant extracts act as fumigants, translaminars as well as antifeedant and nutrition inhibitors (McEwen *et al*. 2001). According to Pascual-Villalobos and Robledo's experiments (1998), some combinations of plant species,

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such as *Digitalis* sp. and *Psoralea* sp. have toxic properties and other poisonous plants such as *Dathura* sp. and *Daphne* sp. can be used for pest control (Pascual-villalobos and Robledo 1998). Botanical insecticides derived from plants of Meliacea have toxic effects on the ladybird *Chilocurus bipustulatus* (Peveling and Ould Ely 2006).

There are a few records in the world about some medicinal plants that have some by-products which may act as plant pesticides. In this research, four plants: *Fumaria parviflora* Lam. (Fumariaceae), *Teucrium polium* L. (Lamiaceae), *Calotropis procera* (Willd.) R. Br. (Asclepiadaceae) and *Thymus vulgaris* L. (Lamiaceae) have extracts evaluated as herbal compounds against cotton whitefly.

Germander, *T. polium*, is one of the aromatic and medicinal plants of the Lamiaceae family that grow naturally in most parts of Iran. The flowering branches and leaves of these plants contain some essential oils that have insecticide properties (Koschier and Sedy 2003; El-Shazly and Hussein 2004; Mahdavi-Arab *et al*. 2008).

Th. vulgaris is another medicinal plant belonging to the Lamiaceae family. It also grows in most parts of Iran. The essential oils and extracts of different species of this plant have insecticidal properties (Hummelbrunner and Isman 2001; Taghizadeh-Sarokalaii and Moharramipour 2010). Hummelbrunner and Isman (2001) tested ten natural compounds such as usenol, on the cotton leaf worm (*Spodoptera litura*). They found that thymol is a toxic component for this pest.

The fumitory plant *F. parviflora* is a medicinal plant that belongs to the Fumariaceae family. This plant grows in the region of Sarcheshme (a suburb of Rafsanjan, south of Iran). The essential oils and plant extracts of this plant also have insecticidal properties (Mahdavi-Arab *et al*. 2008).

The swallow wort plant, *C. procera*, is one of the medicinal plants of the Asclepiadaceae group. It can be found growing naturally in the southern parts of Iran; such as in Haji Abad, Bandar Abbas, and Ourzoeiyh. The essential oils and plant extracts of this plant have insecticidal properties (Mahdavi-Arab *et al*. 2008).

Ecotoxicology studies try to combine ecological principles and toxicology together to obtain a more real assessment, than the environmental dangers of pesticides. In this branch of science, the effects of pesticides at higher levels than the individual level are studied. Such levels as populations, communities, and food chains are studied. Toxicological research, on the other hand, studies only the effects of pesticides on the individual level (Rumpf *et al*. 1997; Stark and Danks 2003; Stark *et al*. 2004). For some pesticides which have high non-destructive effects, the use of population statistics, is the ideal method, because it combines lethal effects with non-destructive effects. Therefore, a more accurate measure of the sub-lethal effects of pesticides on pest species is provided (Stark and Wennegren 1995). In this study, we evaluated the sub-lethal effects of *F. parviflora*, *T. polium*, *Th. vulgaris*, and *C. procera* on such biological parameters as longevity and mortality of different cotton whitefly life (CWE) life stages.

MATERIALS AND METHODS

Insect rearing on host plant

Tomato seeds, Var. Bakker Brothers were planted directly in plastic pots (10x10x16 cm) filled with sterile plant growth media [(Bastare Amadeh Giah Arganic (BAGA); Dashte Sabz Atieco. Iran)]. Cotton whitefly adults were collected from a Rafsanjan field and transferred onto 2–4 tomato leaves in a greenhouse (Department of Plant Protection – Vali-e-Asr Univ.). Adults of the same age were collected from red-eye pupae and moved to separate plant cages. These adults were used in all the experiments.

Plant extracts and pesticides

Plant extraction

Four medicinal plants belonging to four families were used in this study (Table 1). Plants were collected in their flowering stage from natural habitats in different parts of the Kerman province (south of Iran). Selected parts of plants were left to be air dried for 4–5 days before the dried parts were ground. Twenty gr of plant powder were placed on filter paper and steeped in ethanol (90 ml) and water (210 ml) for 12 hours. The extraction was prepared according to the Soxhlet extraction method (Vogel 1978). Afterwards, rotation was used to reach an extract amount of one third.

Botanical pesticides and pymetrozin

Based on their active ingredient, two insecticides: pymetrozin (Chess®25% WP) and a commercial azadirachtin product (Neemarin EC1500) were examined on the eggs of *B. tabaci* (Table 2).

Table 1. Plant species extracts used on adult *B. tabaci*

Scientific name	Common name	Family name	The parts of the plant used
Fumaria parviflora	fumitory	Fumariaceae	leaves and stem
Teucrium polium	germander	Lamiaceae	leaves
Calotropis procera	swallow wort	Asclepiadaceae	leaves and flowers
Thymus vulgaris	thyme	Lamiaceae	leaves

Table 2. Pesticides used on adult *Bemisia tabaci*

Common name	Commercial name	Formulation
Pymetrozin	Chess	25% WP
Azadirachtin	Neemarin	EC 1500

Leaf dip bioassay

The toxicity of four plant extracts *F. parviflora*, *T. polium*, *C. procera* and *Th. vulgaris*, and the two pesticides azadirachtin and pymetrozin for adults of cotton whitefly were assayed by using the leaf-dip method. For bioassay, we applied five different concentrations in three replicates. Two clear plastic glasses (10 cm diameter, 15 cm height) were put together as a plant cage. The upper one was covered with a fine mesh and the lower one filled with distilled water. Two tomato leaves were dipped in the dilutions for 5 s and put in each cage. After drying the treated leaflets, fifteen same age adults were released into the upper part of cage. Mortality was evaluated after the treated adults were maintained in controlled conditions [(27±2°C, 50±5 relative humidity (RH) and 16:8 h L:D)] for 24 h. The criterion for death was that insects were not able to move properly when their legs were probed with a soft camel-hair brush. The LC_{50} values and 95% confidence limits were calculated from probit regressions by the Probit analysis 2011 software.

Accordingly, the lethal doses of 25 percent of each of the plant extracts and pesticides were calculated during the next 24 hours. For this purpose, the leaflets were dipped in a 25 percent lethal concentration of each of the plant extracts and pesticides for 5 s and were allowed to air dry. Thirty same-aged adults were released on tomato leaflets in the plant cages. After 24 h, all adults were removed from the cages and released into new cages. Then, the longevity of the eggs, nymph, pupae and adults were calculated daily by stereomicroscope and the digital microscope Dino capture. Sex ratios, oviposition period, total fecundity/female, mortality of egg, mortality of nymphs and pupa/day and total mortality of eggs, nymphs and pupae were calculated. The experiments were carried out in a completely randomized design (CRD) with at least four replications in controlled conditions.

Statistical analysis:

The collected data were analyzed by SPSS16 software and the means compared using Duncan's test. The LC_{25} was estimated by probit analysis 2011 software. Graphs were drawn using SigmaPlot 11.0.

RESULTS

Effect of some plant extracts, azadirachtin and pymetrozin on:

Biological parameters

Different biological parameters; pupa ($F_{6, 37} = 4.49$, $p < 0.01$) and adult longevity life table ($F_{6, 31} = 4.47$, $p < 0.01$), sex ratio (female) ($F_{6, 30} = 3.49$, $p < 0.01$), oviposition period ($F_{6, 31} = 7.50$, $p < 0.001$), total fecundity/female ($F_{6, 31} = 8.23$, $p < 0.001$), and the egg longevity variable ($F_{6, 39} = 2.96$, $p < 0.05$) showed significant difference between plant extracts, azadirachtin, and pymetrozin while there was no significant difference between nymph longevity ($F_{6, 37} = 1.08$, $p > 0.05$) and developmental time ($F_{6, 36} = 1.51$, $p > 0.05$) (Table 3).

The results showed that pymetrozin and *T. polium* extracts increased egg longevity but *C. procera* extract and azadirachtin decreased it compared to the controls.

The minimum nymphal longevity (10 days) and maximum (12.49 days) was observed when using the *T. polium* and *C. procera* treatment, respectively. The means of the nymphal period did not show significant differences between plant extracts and pesticides.

Pupal longevity showed significant difference between treatments in comparison with the control ones. The *C. procera and T. polium* treatments had the maximum and minimum of differences.

The extract from the herbal compound *C. procera* and pymetrozin had the least and the most effect on adult longevity, respectively, and showed significant differences in comparison with the control treatments.

The results showed that *C. procera* extract and pymetrozin increased developmental time but they did not show significant differences in comparison with the controls.

The use of the *T. polium* extract and pymetrozin decreased the sex ratios (female to male). This was a significant difference compared to the controls.

T. polium and *C. procera* extracts increased the oviposition period and total fecundity/female while azadirachtin and pymetrozin decreased these parameters. There were no significant difference between parameters while there were significant difference in comparison with the controls.

The mortality rate of different life stages

The results showed a significant difference only concerning the mortality rate of the eggs ($F_{6, 39} = 6.71$, $p < 0.001$) and the total mortality of the eggs ($F_{6, 39} = 6.71$, $p < 0.001$), between plant extracts azadirachtin and pymetrozin. While there were no significant differences between nymph mortality ($F_{6, 39} = 1.90$, $p > 0.05$) and pupa mortality ($F_{6, 32} = 0.35$, $p > 0.05$), total mortality of nymph ($F_{6, 39} = 1.87$, $p > 0.05$) and pupa ($F_{6, 30} = 0.12$, $p > 0.05$) (Table 4).

The maximum mortality rate was observed in eggs and pupae when pymetrozin and *F. parviflora* applications were used. The maximum mortality was observed in nymphs when *F. parviflora* and *T. polium* extract applications were used.

DISCUSSION

Based on these results, pymetrozin increased egg longevity, developmental time, mortality rate of egg and pupa and total mortality of egg while it decreased adult life longevity, sex ratios, oviposition period and total fecundity/female. Of all the extracts, pymetrozin had the most effect on these biological parameters of *B. tabaci*. The fumitory extract increased the mortality rate of eggs, nymphs and pupas as well as the total mortality of eggs, nymphs and the pupal duration. These results indicated that fumitory extract could decrease population density of pests almost as much as pymetrozin, though the permanence of the plant extract is less than the insecticide. It is also important to note the lethal effect of the fumitory extract on the different life stages of the pest. Ateyyat *et al.* (2009) reported that the highest percentage of mortality of eggs cotton whitefly (CWF) (20%) was caused

Table 3. Comparison of means related to biological parameters of: *Bemisia tabaci* treated with plant extracts and pesticides

Variable	The control	*Th. vulgaris*	*C. procera*	*T. polium*	*F. parviflora*	Neemarin	Pymetrozin
Egg longevity	6.890±0.192 ab	7.109±0.090 ab	6.682±0.074 b	7.358±0.150 a	7.291±0.200 ab	6.734±0.170 b	7.497±0.257 a
Nymph longevity	11.230±0.354 a	11.305±0.494 a	12.494±0.736 a	10.004±1.167 a	11.674±0.609 a	12.362±0.894 a	12.252±0.951 a
Pupa longevity	3.960±0.318 abc	3.684±0.330 bc	5.2680.476± a	2.692±0.367 c	4.472±0.543 ab	4.231±0.457 ab	3.652±0.392 bc
Adult longevity	6.567±0.701 a	4.050±0.445 b	4.638±0.527 b	4.428±0.316 b	4.246±0.342 b	3.625±0.125 b	3.282±0.526 b
Developmental time	21.555±0.652 a	21.353±0.638 a	23.693±0.600 a	21.157±0.491 a	22.220±0.892 a	22.213±1.560 a	22.775±1.058 a
Sex ratios (female)	0.494±0.036 a	0.243±0.080 b	0.348±0.048 ab	0.180±0.051 b	0.356±0.069 ab	0.472±0.027 a	0.241±0.073 b
Oviposition period	4.829±0.727 a	1.563±0.284 b	2.247±0.471 b	2.500±0.327 b	2.046±0.363 b	1.041±0.150 b	1.297±0.250 b
Total fecundity/female	26.440±5.248 a	6.587±2.280 b	8.936±2.245 b	7.392±1.379 b	6.080±1.649 b	2.958±0.587 b	3.651±1.917 b

Means within a row and in the same stage followed by the same lowercase letter (a, b, c, d) are not significantly different (Duncan̓s test, $p > 0.05$)

Table 4. Comparison of means related to mortality of the different life stages of: *B. tabaci* treated with plant extracts and pesticides

Variable	The control	*Th. vulgaris*	*C. procera*	*T. polium*	*F. parviflora*	Neemarin	Pymetrozin
Mortality of egg duration	0.000±0.000 b	0.126±0.067 b	0.090±0.058 b	0.041±0.023 b	0.135±0.067 b	0.010±0.010 b	0.522±0.129 a
Mortality of nymph duration	0.000±0.000 b	0.247±0.077 ab	0.238±0.075 ab	0.420±0.100 a	0.468±0.133 a	0.339±0.159 ab	0.304±0.137 ab
Mortality of pupa duration	0.130±0.013 a	0.191±0.083 a	0.148±0.043 a	0.225±0.195 a	0.336±0.142 a	0.152±0.064 a	0.226±0.122 a
Total mortality of egg duration	0.000±0.000 b	0.126±0.067 b	0.090±0.058 b	0.041±0.023 b	0.135±0.067 b	0.010±0.010 b	0.522±0.129 a
Total mortality of nymph duration	0.000±0.000 b	0.224±0.072 ab	0.227±0.077 ab	0.417±0.100 a	0.433±0.137 a	0.337±0.160 ab	0.260±0.127 ab
Total mortality of pupa duration	0.130±0.013 a	0.155±0.058 a	0.102±0.025 a	0.179±0.164 a	0.238±0.152 a	0.151±0.067 a	0.145±0.122 a

Means within a row and in the same stage followed by the same lowercase letter (a, b, c, d) are not significantly different (Duncan̓s test, $p > 0.05$)

by the extract of *Euphorbia hierosolymitana*. Al-mazra'awi *et al*. (2009) evaluated insecticidal and repellent activities of medicinal plant extracts against the sweet potato whitefly, *B. tabaci*. They reported that the highest egg mortality percentage was 33% when the *Alkanna strigosa* extract was used. In this research, the fumitory extract with a 13% mortality, had the highest casualties at the egg stage.

Germander extracts increased egg longevity, mortality of nymph duration and total mortality of nymph duration while germander extracts decreased the sex ratio, and nymphal and pupa longevity and developmental time. These results indicate that this extract not only caused death at the immature stages but also caused shorter life duration and decreased female density. Based on the research of Ateyyat *et al*. (2009), *Lepidium sativum* extract with 71% casualties, caused the highest mortality on early stage nymph. Al-mazra'awi *et al*. (2009) demonstrated that extracts from the plants *Ruta chalepensis*, *Peganum harmala* and *A. strigosa* were the most promising extracts for the control of *B. tabaci* resulting in more than a 50% mortality of the immature stages. In this research, fumitory and germander extracts with quantities of 46% and 42%, respectively, caused the highest nymph mortality.

Swallow wort extract increased nymph and pupa longevity and developmental time, and decreased egg longevity, mortality of nymph and pupa, and total mortality of nymph duration. These results indicated that Swallow wort extract had less effect on egg longevity, nymph longevity and the mortality rate of the different stages, but it had more effect on adult longevity, oviposition period and total fecundity/female.

Thyme extract showed different effects on the studied parameters. The insecticide azadirachtin decreased oviposition period and the total fecundity/female parameters more than the others. Such results may be related to the permanence effect of azadirachtin. Al-mazra'awi *et al*. (2009) reported that thyme extract did not show any mortality at the egg stage while it caused a 32% mortality at the nymph stage. In the present research thyme caused a 12% and 24% egg and nymph mortality, respectively.

No report has ever been made of the effects of these extracts as herbal insecticides (except thyme) on CWF. Thus, this study is the first report ever. Some authors have worked on plant extracts other than these.

It seems that differences may be related to the extraction method or laboratory conditions.

According to these reports and this research, herbal compounds caused more mortality on the egg and nymph stage than on the pupal stage. These mortality results may be related to the resistance of the pupal stage in comparison with other stages.

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