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The role of phenols in the influence of herbal extracts from *Salvia officinalis* L. and *Matricaria chamomilla* L. on two-spotted spider mite *Tetranychus urticae* Koch

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Abstract: Extracts prepared from 2 medicinal herbs (common sage *Salvia officinalis* L. and German chamomile *Matricaria chamomilla* L.) were used in this study for biological control of spider mites. Their effect on mortality, fecundity, and development of *Tetranychus urticae* Koch on English ivy (*Hedera helix* L.) was studied under laboratory and greenhouse conditions. The extracts were prepared by soaking fresh *Salvia* leaves or dry *Matricaria* flowers in ethanol for 24 h, followed by evaporation, and finally suspension of the pellets in water. Full extracts and extracts without phenols were tested. The *Salvia* extracts had a higher acaricidal activity than *Matricaria* extracts, but the toxicity of both extracts to spider mite eggs was low. The toxic effect of full *Salvia* extracts on larval stages and females of *T. urticae* was evident. More than 50% of larvae and females were killed by this treatment in 4 days. Besides, total fecundity of survived females was evidently lower. Mortality and low fecundity after full *Salvia* extract application decreased the spider mite population by 76%.The toxicity of *Salvia* extracts after removal of phenols strongly declined, so the negative effect of the full *Salvia* extract on spider mites was connected with a high concentration of phenolic compounds. The toxic effect of *Matricaria* extracts in all tests was much lower and no evident role of phenolic compounds in its extracts was observed.

Keywords: *Salvia officinalis*, *Matricaria chamomilla*, plant extracts, acaricidal activity, *Hedera helix*, *Tetranychus urticae*

INTRODUCTION

Many studies have shown that herbal extracts can be used for biological control, as natural insecticides and acaricides. They are usually rich in secondary metabolites, such as alkaloids, phenols or terpenoids. These substances are known from their repellent, antifeedant, ovicidal, and killing action against arthropod pests (Smith 1989).

Phenolic compounds play an important role in herbivore-host plant interaction (Bi & Felton 1995). Most of them are known as feeding deterrents. Plants with a high concentration of phenols are often less attractive hosts for many herbivorous insects and mites than plants with a low content of these secondary metabolites.

An increased level of phenolic compounds was detected in many cases in response to herbivore attack and is regarded as a defence reaction of the plant. Similar reactions of various host plants have also been observed after their infestation by spider mites (Tomczyk 2002), which suggests the importance of phenolic compounds in defence mechanisms of plants against these pests.

Also terpenoids are bioactive chemicals, which can be used for pest control. The insecticidal and acaricidal activity of many plant extracts is connected with a high concentration of essential oils (Chiasson et al. 2001; Isman et al. 2010). During the last 10 years, acaricidal activity of many different plants, rich in secondary plant metabolites, was widely tested. The acaricidal activity of *Artemisia annua* L. extracts, prepared from various plant parts and with various solvents, was tested for *Tetranychus cinnabarinus* Bois. (Zhang et al. 2008). The biological activity of acetone leaf extract of *A. annua* exhibited the highest contact toxicity against *T. cinnabarinus.* A study of acaricidal activity of *Juglans regia* extracts against *T. viennensis* Zacher and *T. cinnabarinus* showed that mite mortality was much higher when petroleum ether was used for extraction instead of methanol, chloroform, ethyl acetate or distilled water (Wang et al. 2007). Essential oils from sage (*Salvia*) and peppermint (*Mentha*), used as fumigants, are toxic to adult *T. urticae* and its predator *Phytoseiulus persimilis* A.-H. (Choi et al. 2004).

Flower extract from *Matricaria chamomilla* L. seems to be a promising treatment for the control of cattle fever tick (*Rhipicephalus annulatus* Say) in field conditions because of its negative influence on female fecundity and survival (Pirali-Kheirabadi & Razzaghi-Abyaneh 2007). Rasikari et al. (2005) screened the contact toxicity of crude leaf extracts of 67 plant species from 6 subfamilies of the Australian Lamiaceae against *T. urticae* and found that plants from 5 subfamilies showed acaricidal activity. Shi et al. (2006) described the toxic effect of *Kochia scoparia* L. for various species of spider mites. A negative influence of essential oils from several medicinal plants (*Mentha spicata* L., *Thymbra spicata* L., *Lavendula stoechas* L.) on *T. cinnabarinus* has been recently described by Sertkaya et al. (2010).

Beside contact toxicity, plant extracts can have other negative effects on mites. Some of them show a high level of repellency, affect female fecundity or act as development inhibitors. Crude extracts from leaves of wild tomato (*Lycopersicon hirsutum* L.) exhibit both toxicity and/or repellency to *T. urticae*, depending on the solvent used for extraction (Antonious & Snyder 2006). Increased mortality, high repellency as well as reduction of the total number of eggs laid by 2 mite species (*T. urticae* and *Eutetranychus orientalis* Klein) were observed as an effect of the application of extracts prepared from *Origanum majorana* L. (= *Majorana hortensis* Moench) and *Rosmarinus officinalis* L. (Amer et al. 2001). Extracts from various species of *Capsicum* affect *T. urticae* survival and are highly repellent (Antonious et al. 1997). In Israel, extracts of 29 local plant species were tested as potential bio-acaricides to control *T. cinnabarinus.* Mortality, repellency, and number of eggs laid were assessed. The mortality of mites was not very high but 6 best extracts caused more than 75% repellency (*Allium sativum* L., *Capparis spinosa* L., *Cupressus sempervirens* L., *Lupinus pilosus* L, *Rhus coriaria* L., and *Tamarix aphylla* L.). Extracts from 16 plants were found to cause a significant reduction in female fecundity of *T. cinnabarinus*

(Mansour et al. 2004). Fruit and leaf extracts from *Capparis aegyptia* L., prepared with various solvents, also decreased the fecundity of *T. urticae* (Hussein et al. 2006). Reproduction of *T. urticae* significantly decreased after treatments with extracts of *Gardenia jasminoides* Ellies, *Ficus carica* L., and *Albizia coreana* Nakai. A slight repellent activity of some plant extracts against *T. urticae* was also observed (Kim et al. 2005).

The repellent effect of water extracts of *Allium sativum* and *Urtica dioica* Linn on *T. urticae* was observed by Dąbrowski & Seredyńska (2007). Ethanol extracts obtained from both leaves and seeds of *Datura stramonium* L. significantly reduce the fecundity of *T. urticae* females, while increase their mortality and repellency on bean leaves (Kumral et al. 2010). Leaf and root extracts from *Asphedolus aestivus* Brot. have a potential acaricidal activity since they inhibit the oviposition and egg hatching of *T. cinnabarinus* (Genosoylu 2007). The biology of *T. urticae* feeding on bean leaves is also affected by pyrrolizydine alkaloids extracted from *Lithospermum canescens* (Michaux) Lehm. (Pietrosiuk et al. 2003).

Our previous study showed a reduction of fecundity of *T. urticae* females and their lower feeding activity on *Hedera canariensis* L. treated with *Salvia* extracts prepared from dry and fresh leaves (Kawka & Tomczyk 2002).

Crude extracts prepared from various plants can be more effective against pests, as compared to individual compounds, because chemicals may interact synergistically or additively (Hay et al. 1994; Nelson & Kursar 1999; Akhtar & Isman 2003; Sakunwarin et al. 2004).

The aim of the study was to compare the effect of *S. officinalis* and *M. chamomilla* extracts on *T. urticae,* feeding on English ivy (*Hedera helix* L.), and to check the role of the phenolic fraction in the negative influence of the extracts on mite survival, female fecundity, and development of the spider mite population.

MATERIAL AND METHODS

Host plants

Experiments were conducted under greenhouse and laboratory conditions on English ivy plants (*H. helix* cv. Melissa), as hosts for *T. urticae* Koch. The plants were treated with *Salvia* and *Matricaria* extracts to check their influence on mite mortality, fecundity and development.

Extract preparation and fractionation

Homogenized fresh leaves of *S. officinalis* (100 g) and dry flowers of *M. chamomilla* (20 g) were mixed separately with 1000 cm^3 of 96% ethanol and left for 24 h in a dark place to extract secondary metabolites. After filtration, ethanol was removed from the extracts by evaporation at room temperature for about 72 h. The resultant pellets were suspended in 1000 cm^3 of distilled water and used for tests. They were applied directly for treatments or after removal of phenolic fractions. Phenols were removed with polyvinylpolypyrrolidone (PVPP). Extracts with PVPP were left in a dark and cold place for 24 h, then filtrated, and used for the tests.

Influence of extracts on mite mortality

Thirty individuals of each developmental stage of *T. urticae* (eggs, larvae or adult females) were placed on separate leaves and closed in Munger cages. In the case of eggs, before the experiment started, 3-4 young females were placed in the cage for 24 h to lay eggs, then females were removed, and 30 eggs were left on each leaf. Ten leaves for each stage in each extract combination were prepared (10 replications). The cages were transferred to the growth chamber (SANYO) with 23°C, humidity 60%, and day length 16 h.

The influence of extracts on egg mortality was checked after 10 days, whereas mortality of the other stages was assessed after 7 days. Unhatched eggs and dead specimens of mites were counted.

Influence of extracts on fecundity of mite females

The leaves of ivy used in the laboratory tests were dipped in full and phenol-free extracts of *Salvia* and *Matricaria*. Control leaves were dipped in distilled water. When the leaves were dry, they were placed in Munger cages and used for tests with spider mites.

Young, inseminated females of *T. urticae* taken from the culture on ivy were individually placed on the leaves of ivy treated with full extracts of *Salvia* and *Matricaria*, extracts without phenols, or distilled water. The experiment started with 40-50 females for each combination, but not all of them survived to the end of the experiment. Every day, to the end of female life, the eggs were counted and removed. Females were transferred to new leaves every 4-5 days. Because of their high mortality in some combinations, average fecundity was calculated for 20 survived females of every test (20 replications).

Influence of extracts on development of the mite population

Experiments were conducted in the greenhouse on young ivy plants planted in pots. At the beginning of the experiment, every plant was at the stage of 15 leaves. The initial population of *T. urticae* was 5 females per plant. In every combination, 4 plants were used (4 replications). Plants were sprayed with extracts a week before infestation with mites. The treatment was repeated twice, every 7 days. Spider mites were counted 3 weeks after plant infestation. From every plant, 5 leaves were collected (20 leaves in each combination).

Statistical methods

One-way ANOVA was used and significance of differences was determined by Fisher's test with a significance threshold of 0.05.

RESULTS

Influence of extracts on mite mortality

Egg mortality after spraying of ivy leaves with both extracts (Fig. 1), was only slightly higher than after control spraying (with distilled water), but the differences were significant ($F_{2.27} = 4.63$; $P = 0.014$ for *Salvia* and $F_{2.27} = 7.07$; $P = 0.003$ for

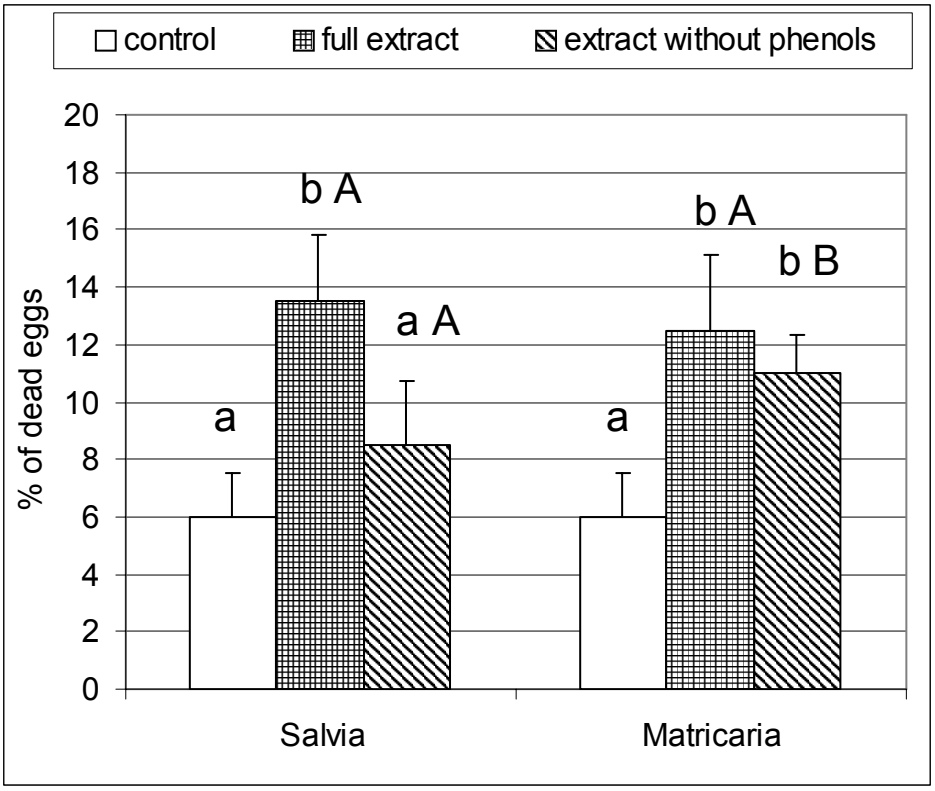

Fig. 1. Mortality of *Tetranychus urticae* eggs on ivy leaves treated with herbal extracts (mean ± SE). Small letters above of the error bars denote differences between extracts of the same plant, while capital letters denote differences between plants

Matricaria). Full *Salvia* extracts were more effective than the extracts without phenol fraction, in contrast to the *Matricaria* extracts. No significant difference was found in the toxic influence of extracts on mite eggs between full extracts of *Salvia* and *Matricaria* ($F_{1.18} = 0.30$; $P = 0.5920$).

Mortality of juvenile stages of *T. urticae* on ivy treated with *Salvia* and *Matricaria* (Fig. 2) shows a high toxicity of full *Salvia* extracts to larvae ($F_{2.27} = 33.75$; $P < 0.0001$). Almost 56% of larvae were killed by this treatment within 4 days. The toxicity of *Salvia* extracts without phenols was only half as high as compared to full extracts, but still significantly higher as compared to the control treatment. Mortality of larvae on ivy leaves treated with *Matricaria* extracts was 34% in the case of full extracts and 30% in the case of extracts without phenols, and significantly differed from the control ($F_{2.27} = 12.17$; $P = 0.0005$). However, no differences were found between the full and phenol-free extracts. The mortality of larvae after spraying of

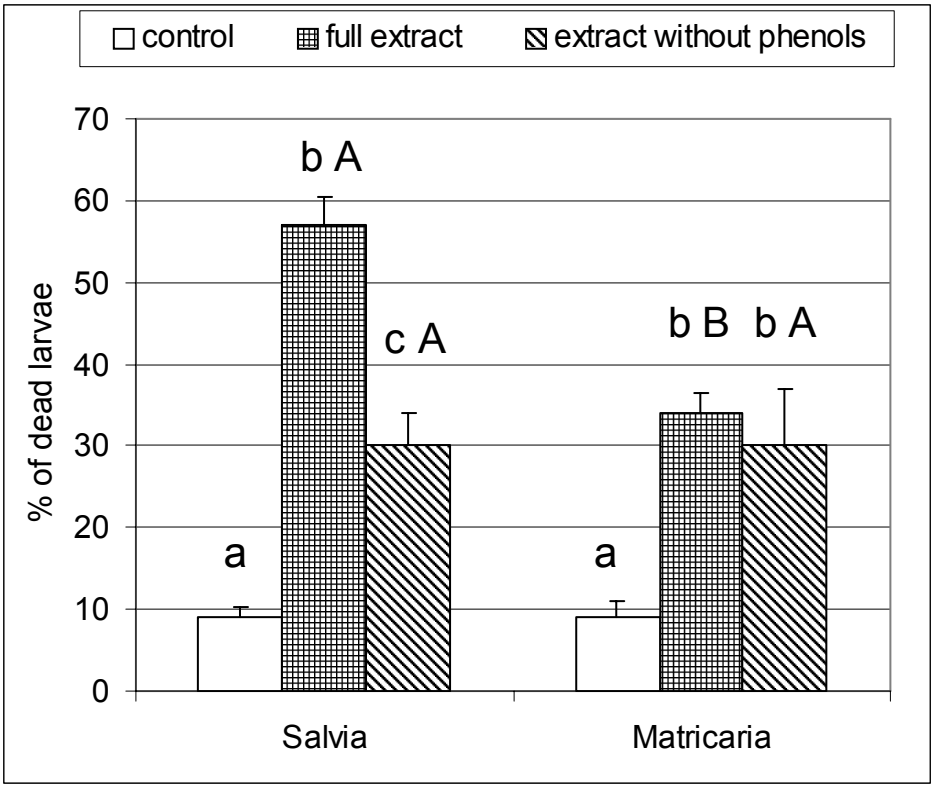

Fig. 2. Mortality of juvenile stages of *T. urticae* on ivy leaves treated with herbal extracts (mean ± SE). Small letters above of the error bars denote differences between extracts of the same plant, while capital letters denote differences between plants

the plants with the full *Salvia* extract was significantly higher as compared to the full *Matricaria* extract ($F_{2.27} = 59.07$; $P < 0.0001$).

Tetranychus urticae females, feeding on ivy leaves, were strongly sensitive to the full extract made from *Salvia* leaves (Fig. 3). More than 50% of females were dead 4 days after treatment. Differences between the effects of the full extract and both the extract without phenols and the treatment with water (control) were significant ($F_{2.27} = 10.01$; $P = 0.0003$). Removal of phenols from the extract decreased its toxicity to females.

The full extract prepared from *Matricaria* was much less toxic to *T. urticae* females than the full *Salvia* extract (Fig. 3), but in comparison with distilled water, the mortality of females feeding on treated leaves was significantly higher ($F_{2.27} = 6.12$; $P = 0.012$).

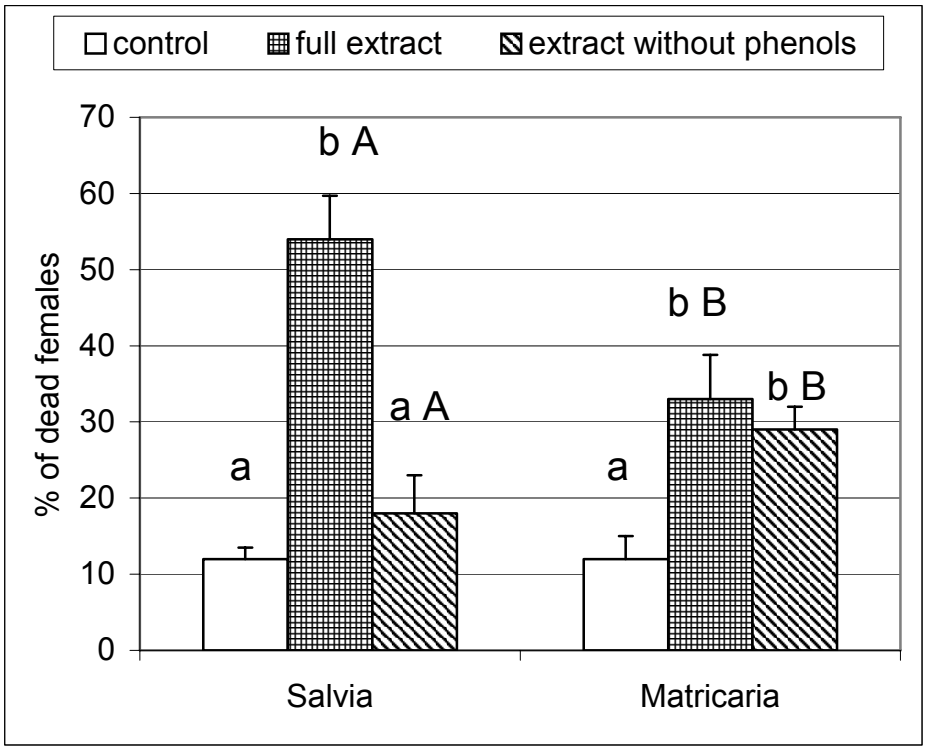

Fig. 3. Mortality of *T. urticae* females on ivy leaves treated with herbal extracts (mean ± SE). Small letters above of the error bars denote differences between extracts of the same plant, while capital letters denote differences between plants

Influence of extracts on fecundity of mite females

Total fecundity of *T. urticae* was strongly affected by extracts prepared from plants of both species of herbs (Fig. 4). The number of eggs laid by females feeding on ivy leaves treated with a full extract of *Salvia*, was more than 3-fold lower as compared to the control (11 and 36, respectively). Removal of the phenolic fraction from the extracts lowered their toxic influence on female fecundity, but still females laid only half as many eggs than on leaves treated with distilled water. The differences between both extracts and the control were significant ($F_{2.27}$ = 12.03; P = 0.0001). The effect of *Matricaria* extracts was less evident but their application on ivy leaves also strongly decreased female fecundity (Fig 4). The differences between all combinations are significant ($F_{2.27}$ = 11.15; P = 0.0001).

Full extracts of *Salvia* were significantly more effective in decreasing of *T. urticae* fecundity, as compared to full *Matricaria* extracts ($F_{2.27}$ = 7.08; P = 0.03) in contrast to phenol-free extracts ($F_{2.27}$ = 1.68; P = 0.089).

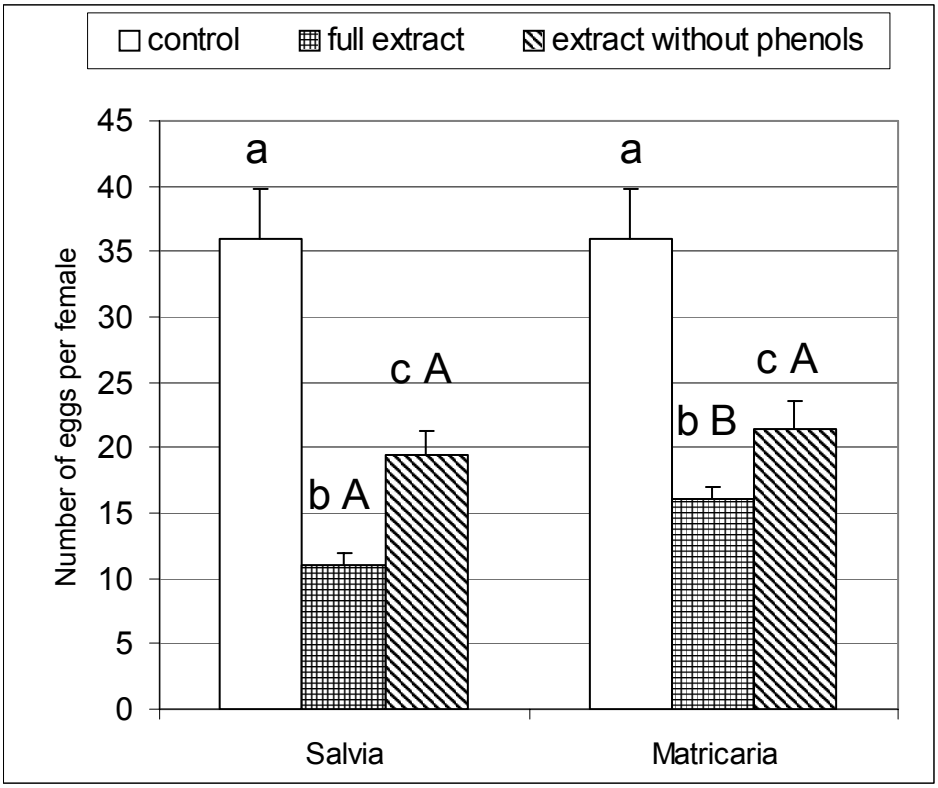

Fig 4. Fecundity of *T. urticae* females on ivy leaves treated with herbal extracts (mean ± SE). Small letters above of the error bars denote differences between extracts of the same plant, while capital letters denote differences between plants

Influence of extracts on development of the mite population

The development of the spider mite population on ivy plants (Fig. 5) was strongly inhibited by both *Salvia* extracts: full and without phenols. However, the extract without phenols was less effective. After 3 weeks of the experiment, on the leaves treated with full extracts, the density of the spider mite population reached 12 mites per leaf, which was only 24% of mite density on the control plants, while 39% on the leaves treated with the extract without phenols ($F_{2.57} = 14.74$; $P < 0.0001$).

Extracts made from *Matricaria* had a small effect on the development of *T. urticae* on ivy in greenhouse conditions. After spraying of the plants with both extracts, the spider mite population decreased by about 17% in relation to the mite population developing on the plants treated with distilled water but the difference was not significant ($F_{2.57} = 1.47$; $P = 0.6235$). No significant difference was found between the effectiveness of full and phenol-free *Matricaria* extracts.

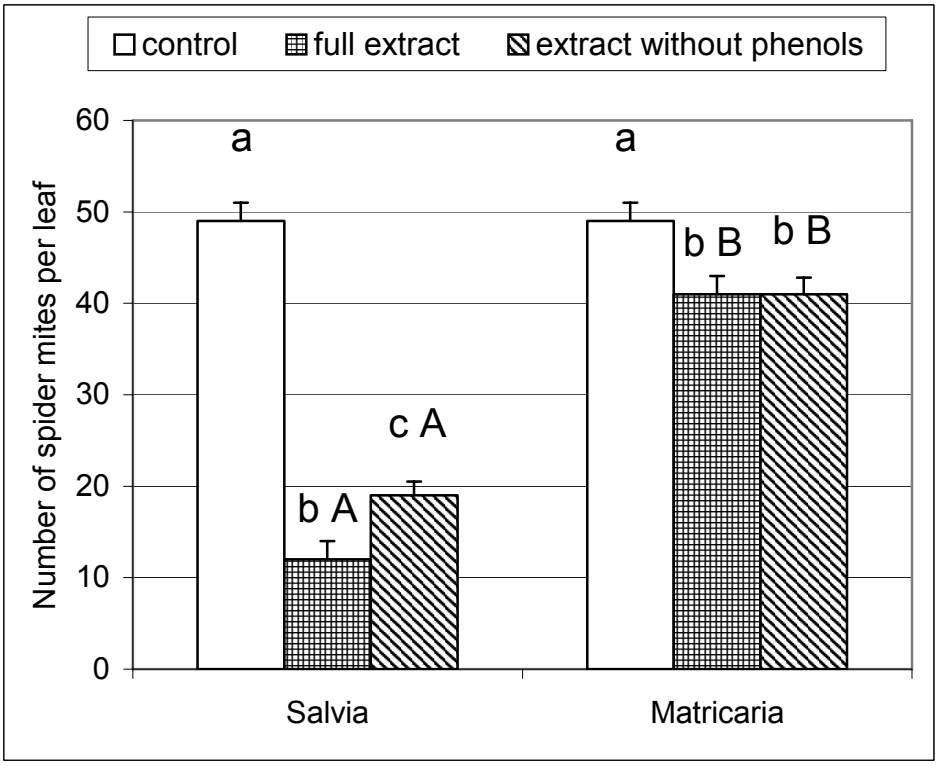

Fig. 5. Development of the *T. urticae* population on ivy after treatment with herbal extracts (mean ± SE). Small letters above of the error bars denote differences between extracts of the same plant, while capital letters denote differences between plants

DISCUSSION

It is evident from the presented data that extracts prepared from the leaves of *S. officinalis* have a stronger negative effect on spider mites feeding on ivy, as compared to extracts made from flowers of *M. chamomilla.* The only exception was their ovicidal activity, which was low for all extracts. Full *Salvia* extracts were toxic to the mobile stages of spider mites: juveniles and females. Choi et al. (2004) also described a low ovicidal activity and a toxic effect of secondary metabolites from *Salvia* leaves on adult mites. Those authors studied the toxic effect of essential oils, but neglected the influence of other active substances. Essential oils can be responsible for a high toxicity of many plant extracts to mites, e.g. extracts prepared also from some other species of Labiatae, *Origanum onites* L., *Thymbra spicata* L., or *Mentha spicata* (Mansour et al. 2004; Sertkaya et al. 2010). A strong lethal effect on spider mites

(up to 100% mortality) was recorded for extracts prepared from plants containing alkaloids, such as *Artemisia annua* (Zhang et al. 2008), *Lycopersicon hirsutum* (Antonious & Snyder 2006) or *Datura stramonium* (Kumral et al. 2010). In our study, the maximum spider mite mortality in the laboratory was 59%, after application of crude ethanolic *Salvia* extracts. Their application in the greenhouse strongly reduced (by 76%) the *T. urticae* population on ivy plants.

The negative influence of plant extracts on the mites is not only connected with their contact toxicity but also with the repellency or the influence on mite development or female fecundity. In our experiments, fecundity of *T. urticae* was affected by both *Salvia* and *Matricaria* extracts. Several authors observed a reduction in the number of eggs laid by mite females on plants treated with plant extracts (Mansour et al.2004; Kim et al. 2005), even though contact toxicity of the extract was relatively low (Amer et al. 2001; Gencsoylu 2007). Methanolic extracts from various species of *Capsicum* used against *T. urticae* also did not result in high mite mortality (the greatest mortality was 45%, when the fruit extract of *C. annuum* was used) but they may have a great potential for repelling spider mites (Antonious et al. 1997). Plant extracts with a high level of essential oils repel spider mites (Isman et al. 2010).

Our data suggest that the negative effect of *Salvia* on spider mites is connected with a high concentration of phenolic compounds. Phenols were especially toxic for adult mites, increasing their mortality. After removal of these compounds by PVPP, the toxic effect of *Salvia* extracts was decreased, much more strongly for females than for juvenile stages. The fecundity of the females that did not die after application of the extracts was also less affected by extracts without phenols as compared to full *Salvia* extracts. In an earlier study we also observed a negative influence of *Salvia* extracts on *T. urticae* fecundity (Kawka & Tomczyk 2002).

Our data indicate that the negative effect of the *Salvia* extracts was not only due to the contact toxicity for mobile stages of *T. urticae,* especially for the females. The extracts strongly affected also the fecundity of the females that survived after an extract application. The phenolic fraction played an important role in the negative influence of the *Salvia* extract on *T. urticae.* The extract without the phenolic fraction, however, still strongly affected the survival of juvenile stages and female fecundity. This can be connected with a high concentration of terpenoids in *Salvia* leaves (Choi et al. 2004). As it was shown by Sertkaya et al. (2010), essential oils present in the extracts of many herbs, also in *Salvia officinalis,* showed toxic, repellent, and ovicidal action against spider mites.

Hussein et al. (2006) found that solvents used for extract production have an important meaning for their acaricidal activity. Ethyl acetate extract of leaves and fruits from *Capparis aegyptia* was more effective for eggs and females of *T. urticae* than ethanolic extracts. Mortalities of *T. viennensis* and *T. cinnabarinus* caused by concentrated leaf extracts of *Juglans regia* prepared by chloroform, ethyl acetate, methanol, and distilled water were significantly lower than by petroleum ether (Wang et al. 2007). It is possible that other solvents, if used in our study, could increase the toxic effect of the extracts, especially of the extract prepared from *Matricaria*. Ethanolic extracts are, however, often used for extraction of biological active substances, both for medical and plant protection purposes.

The way of extract preparation for our experiments was similar to other studies (Mansour et al. 2004; Wang et al. 2007). An increase in extract concentration as well as the use of another solvent could probably increase their acaricidal activity, as it was observed by Wang et al. (2007).

A majority of studies on the effectiveness of plant extracts for mite control were conducted in laboratory conditions on leaf fragments. In our study, the negative effect of *Salvia* extracts on mortality and fecundity of spider mites was confirmed in a greenhouse experiment, where development of the *T. urticae* population was studied on treated and untreated plants. A lack of phenols in the *Salvia* extract resulted in a decrease in its negative effect on the mite population.

From all tests with extracts prepared from *M. chamomilla* it is clear that their effect on spider mites in the applied concentrations was not satisfactory, as compared to *Salvia* extracts. Removal of phenolic substances from the full *Matricaria* extract did not change its toxicity to spider mites. The increased mortality of mobile mite stages and the decreased fecundity of females, as compared to the control, was in this case probably not connected with phenols but with terpenoids. Extraction of chamomile flowers with other solvents (even in the same concentration) may improve their acaricidal activity, since terpenoids are known from their repellent and toxic effect on spider mites (Choi et al 2004; Isman et al. 2010; Sertkaya at al. 2010).

From the obtained data it can be concluded that:

1. Ethanolic extract of fresh leaves of *Salvia* can decrease the *T. urticae* population on ivy, because of its toxic effect on mobile stages as well as a negative influence on female fecundity.
2. The phenolic fraction plays an important role in acaricidal activity of the *Salvia* extract against *T. urticae*, because elimination of this fraction decreases the negative influence of the extract on female survival and fecundity. However, the phenol-free *Salvia* extract is still toxic to larvae.
3. Phenols probably are in synergy with other compounds (e.g. with essential oils) in the acaricidal activity of *Salvia* extracts.
4. The important role of the phenolic fraction in the negative influence of *Salvia* extract suggests that a solvent that can improve phenol extraction (e.g. methanol) may also improve the acaricidal activity of *Salvia* extracts.
5. Secondary metabolites from herb plants can be very useful in plant protection, especially in combination with other non-chemical methods. More studies are needed on their negative influence, not only on pests but also on natural enemies.

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