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ORIGINAL PAPER

Extract from the roots of *Saponaria officinalis* as a potential acaricide against *Tetranychus urticae*

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Abstract The two-spotted spider mite (*Tetranychus urticae* Koch.) is one of the most harmful phytophagous pests, dangerous not only due to its fast development cycle and high fertility, but also due to its ability to rapidly develop resistance to active substances of acaricides. It is therefore important to seek new alternative products characterized by a novel mechanism of action while being safe to health at the same time. For this reason, we tested the efficacy of extracts obtained by extraction of the roots of *Saponaria officinalis* in water against all developmental stages of *T. urticae*. The highest sensitivity was found for eggs ($LC_{50} = 0.31\%$ w/v), while adults showed the least significant sensitivity ($LC_{50} = 1.18\%$ w/v). Application of the extract also resulted in an inhibition of oviposition by females ($LC_{50} = 0.91\%$ w/v). The efficacy of extract prepared by maceration of 15 and 30 g of *S. officinalis* roots in one litre of water was verified in one-year greenhouse tests in cucumbers and tomatoes infested by *T. urticae*. Repeated applications of the extract were found to significantly reduce the numbers of two-spotted spider mite individuals on tomato and cucumber leaves, and their counts remained significantly lower compared to untreated plants throughout the observation period (140 days). The extract, prepared from 30 g of the roots in one litre of water, maintained spider mite counts at approximately the same levels as an applied commercial acaricide based on a.i. abamectin. At the same time, it was observed that the extract had a positive effect with respect to the mean weight of the fruits and to the overall yield of tomato and cucumber fruits, compared to untreated plants. In addition, the amounts of substances extracted from the roots of *S. officinalis* using water, as well as the extraction velocity of water-soluble substances, were studied. The amounts of water-extractable substances were found to be directly dependent on the weights of the extracted roots, where the extraction of 15, 30, 60, 80 and 100 g of roots in one litre of water resulted in 7.4, 15.9, 30.6, 38.9 and 49.4 g of dry mass of the substances, respectively, dissolved in one litre of the extract after 24 h. Also, the extraction velocity at ambient temperature was very high. When 30 g of roots was extracted in one litre of water, most of the substances were dissolved during the first 10 min (12.9 g L^{-1}); subsequently, the amounts of dissolved substances kept rising only slightly and stabilized after about 25 min from the beginning of extraction (15.5 g L^{-1}). Given that the root extract is primarily used in the food industry, in traditional medicine and in the cosmetics industry, we can presume that the use of the extract for the protection of vegetables against *T. urticae* is of no concern. Based on our tests, we can propose this extract as a candidate basic substance that may be beneficial for reducing the counts of harmful developmental stages of *T. urticae*.

Keywords Basic substances · *Tetranychus urticae* · Botanical acaricides · Plant extracts · *Saponaria*

Key message

- The two-spotted spider mite (*Tetranychus urticae*) is one of the polyphagous pests that show a fast

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development cycle, high fertility and an ability to rapidly develop resistance to acaricides.

- New acaricidal substances should be sought amongst plants used in the food industry, traditional medicine and in the cosmetics industry.
- The efficacy of extracts obtained from the roots of *Saponaria officinalis* against developmental stages of *T. urticae* was studied.
- Acute toxicity of the extract was found for all developmental stages of *T. urticae*. LC_{50} ranged between 0.3 and 1.2% (w/v) depending on the developmental stage.
- Regular application of the extract on tomato and cucumber plants maintained the counts of *T. urticae* below their threshold of harmful effects.
- The extracts had a positive effect on increased yields of cucumbers and tomatoes.
- The extract from *S. officinalis* provides a high potential for being proposed for authorisation in EU countries as a basic substance.

Introduction

Tetranychus urticae Koch (Arachnida: Acari: Tetranychidae) represents one of the most polyphagous arthropod herbivores, feeding on more than 1100 plant species, including more than 150 of economic value, belonging to more than 140 different plant families (Migeon and Dorkeld 2016). It is a major pest of greenhouse production and field crops, especially in *Solanaceae*, *Cucurbitaceae* (e.g. tomatoes, cucumbers, eggplants, peppers and zucchini) and greenhouse ornamentals (e.g. roses, chrysanthemums and carnations), annual field crops (e.g. bean, maize, cotton and soybeans) and perennial cultures (alfalfa, strawberries and citrus plants) (Cazaux et al. 2014; Van Leeuwen et al. 2015). Computer modelling suggests that with intensifying global warming, the detrimental effects of two-spotted spider mite in agriculture will markedly increase due to accelerated development at high temperatures (Van Leeuwen et al. 2010).

Tetranychus urticae is known for its ability to develop rapid resistance to pesticides. Amongst arthropods, it has the highest incidence of pesticide resistance (Whalon et al. 2016). Chemical control often causes a broad cross-resistance within and between pesticide classes, resulting in resistance to novel pesticides within 2–4 years. Many aspects of the biology of the two-spotted spider mite, including rapid development, high fecundity and haplodiploid sex determination, seem to facilitate rapid evolution of pesticide resistance. Control of multi-resistant mites has become increasingly difficult (Khajehali et al. 2011; Kwon et al. 2015).

The frequent development of resistance of *T. urticae* to active substances contained in synthetic insecticides, as well as the potential environmental and health-related risks associated with the application of pesticidal substances (Karabelas et al. 2009; Fantke et al. 2012), are the reasons behind the present intensive search for new, suitable alternatives for plant protection with minimum negative impacts.

Highly promising alternatives for plant protection also include the utilization of plant secondary metabolites, synthesized by some plants as part of their natural defensive capacity against pathogens and pests (Isman 2006; Bakkali et al. 2008). As shown by many studies (Isman and Grieneisen 2014), some plant secondary metabolites provide significant pesticidal effects including insecticidal, growth-inhibiting, antifeedant, antiovipositional and repellent effects against insects (Govindarajan et al. 2013; Benelli 2015a, b; Pavela et al. 2013; Pavela 2015a). In general, these substances are obtained from the plant material using a suitable isolation method (Sajfrtova et al. 2013) and are subsequently used as active substances in the so-called botanical insecticides (Bakkali et al. 2008; Isman and Grieneisen 2014). Lately, very intensive research on plant extracts has resulted in discoveries of new insecticidal substances that could be considered suitable for the development of new botanical insecticides (Isman and Grieneisen 2014), including acaricides (Attia et al. 2013, 2015; Pavela 2015c). Extracts from such plants usually contain compounds safe for the health and the environment (Isman 2006; Bakkali et al. 2008; Regnault-Roger et al. 2012, Pavela 2014a, 2016b), and moreover, thanks to their reciprocal synergic relationships (Pavela 2014b, 2015b) and different mechanisms of action (Rattan 2010), it can be expected that resistant pest populations should not develop.

Due to the ever-increasing demand for safe foods in EU countries, and considering the frequently lengthy and difficult authorisation processes associated with legalisation of BI sales (Isman 2015), new legislative options can be used for more natural alternatives to conventional insecticides in the European Union: the European Food Safety Authority is currently evaluating certain botanicals as “low-risk active substances” (LRASs) or “basic substances” (BSs) as defined by (EC) Regulation No. 1107/2009 (see Article 23). Specifically, such active ingredients should not be neurotoxic, immunotoxic or endocrine-disrupting, nor should they be carcinogenic, mutagenic, corrosive or skin sensitisers (Marchand 2015). This should provide clarity and perhaps a shorter regulatory path for botanicals that meet these criteria (Chandler et al. 2011).

In order to introduce new BSs into practice, suitable candidates need to be found. Currently (the beginning of 2016), the first eight BSs have been authorized, amongst which only the extracts based on *Equisetum arvense* L. and *Salix* spp. cortex are prepared from plant materials. It is therefore important to continue finding new suitable candidate BSs that could become suitable substitutes for risky pesticidal substances.

Saponaria officinalis L. (Caryophyllaceae), commonly called soapwort, is native to Europe and Asia and is cultivated throughout the world for its roots, which have found plenty of traditional uses. Its detergent properties have been well known since ancient times, and its main traditional use has been as a soap. As an herbal medicine, it has been used as an expectorant in bronchitis and topically for skin complaints as well as rheumatic disorders (Bisset 1994; Lu et al. 2015). In the food industry, it has been used for the production of traditional halva or turrón and other sweets (Korkmaz and Özçelik 2011).

As found in our previous study, the aquatic extract from the roots of this plant provides an acaricidal effect (Pavela 2016a). However, this extract has not been studied in greater detail. Our study therefore explores the effect of the aquatic extract on the mortality of individual developmental stages of the important polyphagous mite *T. urticae* and on population dynamics in greenhouse trials. In addition, we have determined the method and the time necessary to achieve dissolution of the active substances in water, as well as the effect on the yields and condition of cucumbers and tomatoes grown in the greenhouse.

Materials and methods

Pests

Two-spotted spider mites, *T. urticae* Koch (Acari: Tetranychidae), were obtained from the cultures maintained at the Crop Research Institute (Czech Republic). The two-spotted spider mite used in the experiments was reared on bean plants (*Phaseolus vulgaris* L. var. Carmen) in a growth chamber (22–25 °C; 16 h photoperiod).

Plant material used for extraction

Commercially sold roots were obtained from a company (Byliny Mikes, Czech Republic) engaged in the sale of medicinal plants. The roots were obtained from two-year plants harvested in November 2014, which were adapted after the harvest using standard methods according to European Pharmacopoeia (Wichtl 2004), i.e. they were dried and ground to pieces approximately 0.5 cm long.

Extraction

Determination of the content of substances dissolved in water

Various amounts of the roots of *S. officinalis* (100, 80, 60, 30 and 15 g) were macerated, each time in one litre of drinkable water for 24 h, at ambient temperature (21 ± 1 °C). Subsequently, the extracts were filtered using filter paper. 10 ml of the extract was removed from every sample using a pipette; this amount was subsequently dried for 12 h at 80 ± 1 °C. The unevaporated residue was weighed, and the mass was used to calculate the percentage of substances dissolved in the extract, expressed in the text as % of the extract's dry mass weight in the volume of water (% w/v). The entire experiment was repeated five times.

Determination of the dissolving velocity of the substances in water

30 g of the roots of *S. officinalis* was extracted in one litre of drinkable water at ambient temperature (21 ± 1 °C). During the maceration, 10 ml of the extract was taken in various time intervals (at 5, 10, 15, 20, 30, 60, 120, 200 and 300 min) using an electronic pipette. The % (w/v) of dissolved substances was determined for every collected sample as described above. The entire experiment was repeated five times.

Bioassays

Acute toxicity

The extract was prepared by macerating 30 g of the roots of *S. officinalis* in 1 L of drinkable water. The extraction was done over 30 min at ambient temperature (21 ± 2 °C). The filtered extract was used as the stock solution for subsequent dilution with water in a concentration series. 10 ml of the solution was taken from every diluted solution used for application to determine the content of dissolved substances (using the above-described method). Five concentrations were used for application: 1.9, 1.5, 1.1, 0.7 and 0.3% (w/v).

In order to determine acute toxicity, individual concentrations were applied to bean plants (*Phaseolus vulgaris* L. cv. Aidagold) with a defined number of adults, protonymphs (in the text below nymphs) or eggs. The bean plants were adapted in such a way that they had only one fully developed leaf. A total of 20 adults (age 3–7 days) were introduced onto every leaf 12 h before application using a fine brush, and the number of living adults was ascertained again immediately before application. Eggs or nymphs were prepared as follows: 10 females were allowed to lay eggs on every bean leaf for 12 h. Subsequently, the females were removed, and the eggs were left for 3 days at 21 ± 2 °C. Application then followed. Alternatively, the eggs were left to develop naturally until the birth of the larvae. The nymphs were left on the plants to develop for an additional five days, and then the plants with a defined nymph count were treated using the prepared extracts. The extracts were applied to the plants using a manual electronic atomizer in a dose approximately equivalent to the application of 600 L of water per hectare. Control plants were treated using only water. The experiment was repeated five times.

The plants were placed in a growth chamber (L16:D8, 25.0 ± 1.0 °C). The numbers of adults and nymphs on the plants were determined using a binocular magnifier at 48 h after application. The eggs were left to develop until the birth of the nymphs (for approximately 10 days); those eggs from which no nymphs had hatched were considered dead.

Inhibition of oviposition

The method according to Pavela (2015c) was used to determine the effect of the extract on the inhibition of oviposition by the females, with minor modifications. Five females (3–4 days old) were transferred using a fine brush onto each of the cut bean leaf discs sized 1 cm^{-2}. The leaf discs were obtained from those bean leaves that had been treated identically as described under "Acute toxicity" and after drying of the spray, using a cork borer. The cut discs with the females were placed in Petri dishes with an agar bottom. The females were removed after 48 h, and the laid eggs were counted. Subsequently, the number of eggs was determined for individual concentrations, and the effective concentration causing % inhibition of oviposition by 50 or 90% compared to the control. The Petri dishes were placed in a growth chamber (L16:D8, 25 °C). The experiment was repeated five times.

Determination of the effect of repeated applications on the incidence of T. urticae adults and nymphs, and the yields of cucumbers and tomatoes grown in the greenhouse

The greenhouse experiment was performed in 2015 to verify practical use of the extract obtained from the roots of *S. officinalis* for plant protection against *T. urticae* and to verify the effect of applications on the yields of vegetables grown in the greenhouse.

Seedlings of *Cucumis sativus* L. cv. Paska F1 (Cucurbitaceae) and *Solanum lycopersicum* L. cv. Sláva Porýní (Solanaceae) were planted in the middle of May in an air-conditioned greenhouse. The plants were planted in beds of sandy loam soil (pH 6.7; fertilized before planting with a fertilizer containing N = 7%; P_2O_5 = 12%; K_2O = 10%; MgO = 1% in one dose of 25 g m^{-2}) with a spacing of 80 × 60 cm. A system of fully randomized blocks was used in three repetitions. Every treated variant was composed of 16 plants, of which 10 plants were randomly selected each time and evaluated during the experiment. 10–15 adults of *T. urticae* were introduced onto every plant in the first week of June to ensure uniform infestation of the plants. During the productivity period (from 25 Jun 2015 to 30 Sep 2015), the plants were treated in regular intervals of 12–15 days using extracts prepared from the roots of *S. officinalis* in a dose of 30 or 15 g in one litre of water. The extraction was done for 30 min. The extracts were applied to the plants using a manual electronic atomizer in a dose approximately equivalent to the application of 800 L of water per hectare. The commercial acaricide Vetrimec 1.8 EC (a.i. 18 g L^{-1} abamectin, manufacturer: Syngenta Crop Protection AG, Switzerland) in a concentration of 0.06% (v/v) was applied as a positive control. Control plants were treated using only water.

Cucumber or tomato fruits were harvested once weekly throughout the fruit-bearing period of the plants. The numbers of the fruits and their weight were determined for every observed plant. The following basic yield characteristics were selected for evaluation of the yield: mean number of fruits per plant, mean weight of one fruit and mean total weight of fruits obtained from one tomato or cucumber plant. Besides evaluation of their fertility, *T. urticae* incidence in the plants was monitored as follows: during the vegetation period of the plants, 20 leaves were randomly collected in approximately 10-day intervals and the leaves were used to determine the number of living individuals of *T. urticae*.

Greenhouse temperatures in the trials were maintained between 24.6 and 29.9 °C during the day and between 18.0 and 22.5 °C at night. Relative air humidity ranged between 54 and 79%. The plants were watered regularly using drip irrigation.

Data analysis

The dependence between the number of extracted roots or the time of extraction and the amount of dissolved substances was plotted.

The mortality of adults, nymphs or eggs was corrected by Abbott's formula (Abbott 1925), and this was expressed as mean mortality percentage. The ascertained number of dead individuals was used to estimate lethal concentrations ($LC_{50,90}$) using Probit analysis and associated 95% confidence limits (CI_{95}) for each treatment (Finney 1971). Inhibition of oviposition was calculated according to the formula $I(\%) = [(C-T)/(C + T)] \times 100$, where C = the number of eggs oviposited in the control and T = the number of eggs oviposited in the treated plants. Probit analysis was used to estimate effective concentrations ($EC_{50,90}$) including corresponding CI_{95} values, which caused oviposition inhibition by 50(90)% compared to the control. If the CI_{95} value ranges overlap, the difference between individual $LC(EC)_{50,90}$ concentrations is not significant ($P = 0.05$).

The numbers of *T. urticae* individuals found on average on one leaf of the plants during the vegetation period were plotted in order to evaluate the efficacy of the *S. officinale* root extract applied to tomato or cucumber plants in the greenhouse. The yields, number of fruit/plant of fruits were evaluated by one-way ANOVA and Turkey's honest significant difference (HSD) test ($P \leq 0.05$). (SAS Institute. SAS/STAT User's Guide 2004).

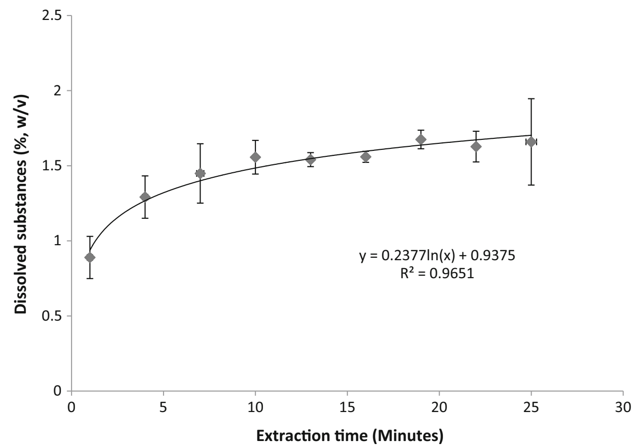

Fig. 2 Extraction velocity of 30 g of *S. officinalis* roots in 1 L of water

Results

Extraction

The amount of substances extracted from the roots of *S. officinalis* using water, expressed as the percentage weight of the dry mass of the substances dissolved in the extract, is shown in Fig. 1. A direct dependence was found between the amount of extracted substances in the water and the weight of the extracted roots. After extraction of 15, 30, 60, 80 and 100 g of roots in one litre of water, 7.4, 15.9, 30.6, 38.9 and 49.4 g of dry mass of the substances were determined in one litre of the extract after 24 h, which corresponds to 0.74, 1.59, 3.06, 3.89 and 4.94% (w/v), respectively.

In addition, the extraction velocity at ambient temperature was very high (Fig. 2). When 30 g of roots was extracted in one litre of water, most of the substances were dissolved during the first 10 min (1.29% w/v); subsequently, the amounts of dissolved substances kept rising only slightly and stabilized after about 25 min from the beginning of extraction (1.55% w/v). The process of releasing of the substances soluble in water was observed for 300 min; after this period, 1.62% w/v was found, which also corresponded approximately to the content of substances released during 24 h of maceration (Fig. 1), where the extract obtained from 30 g of roots contained 1.59% of dry mass of the substances.

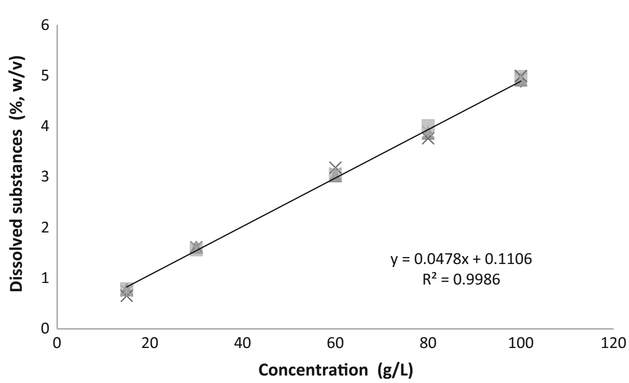

Fig. 1 Amount of substances extracted from the roots of *S. officinalis* using water, expressed as the percentage weight of the dry mass of the substances dissolved in the extract

Acute toxicity

The extract was toxic against all developmental stages of *T. urticae* (Table 1). The highest sensitivity was shown by eggs, with LC_{50} estimated as 0.31% (w/v); however, LC_{90} showed no significant difference (1.31%) from the LC_{90} estimated for the nymphs (1.19%). The least significant sensitivity ($P = 0.05$) was shown by the adults, where $LC_{50(90)}$ was estimated as 1.18 (1.71)%, respectively.

Application of the extract also caused inhibition of oviposition by females, where a concentration of 0.91% resulted in 50% fewer eggs laid compared to untreated plants (Table 1).

Efficacy of the extract on the yield of fruits, and incidence of *T. urticae* in tomato and cucumber plants

One-year greenhouse tests were used to verify the effect of regular application of the extract on *T. urticae* incidence in tomatoes and cucumbers. The mean number of two-spotted spider mite individuals on tomato leaves (Fig. 3) and cucumber leaves (Fig. 4) was significantly lower throughout the observation period (140 days) compared to the untreated plants. An extract prepared from 30 g of roots in one litre of water maintained the counts of two-spotted spider mite at approximately the same levels as an applied commercial acaricide based on a.i. abamectin.

Application of an extract from the roots of *S. officinalis* prepared from 30 g L^{-1} caused a significant increase in the

Table 1 Toxicity and inhibition of oviposition of extract from *S. officinalis* against *T. urticae*

Conc. (%; w/v)	Adults			Nymphs			Eggs			Oviposition		
	Mortality (%) ± SD[a]	LC_{50} $(CI_{95})^b$	LC_{90} $(CI_{95})^b$	Mortality (%) ± SD	LC_{50} $(CI_{95})^b$	LC_{90} $(CI_{95})^b$	Mortality (%) ± SD	LC_{50} $(CI_{95})^b$	LC_{90} $(CI_{95})^b$	Inhibition (%) ± SD[d]	LC_{50} $(CI_{95})^d$	LC_{90} $(CI_{95})^d$
1.9	98.9 ± 2.7	1.18	1.71	100.0 ± 0.0	0.78	1.19	100.0 ± 0.0	0.31	1.31	72.1 ± 7.2	0.91	3.75
1.5	78.3 ± 3.2	(0.76–1.53)	(1.69–2.65)	100.0 ± 0.0	(0.72–0.83)	(1.09–1.36)	100.0 ± 0.0	(0.25–0.35)	(0.38–1.58)	65.5 ± 8.1	(0.68–1.09)	(2.83–4.21)
1.1	49.2 ± 1.9			85.3 ± 5.2			89.3 ± 6.2			53.2 ± 6.9		
0.7	31.8 ± 4.5			48.6 ± 2.8			75.5 ± 4.2			46.5 ± 5.2		
0.3	5.6 ± 0.8			18.3 ± 2.2			49.7 ± 5.5			32.1 ± 8.1		
0.2	0.0 ± 0.0			5.7 ± 1.1			38.2 ± 3.7			12.3 ± 5.6		
Chi[c]		0.517			0.213			0.511			0.885	

[a] Average mortality ± standard deviation (corrected by Abbott)

[b] Concentration LC_{50} (LC_{90}) in % (w/v) causing 50(90)% mortality of adults, nymphs and eggs *T. urticae*. CI_{95}—95% confidence intervals, extract activity is considered significantly different when the 95% CI fail to overlap

[c] Chi-square value, significant at $P < 0.05$ level

[d] Mean inhibition of oviposition in comparison with the control ± standard deviation. Concentration LC_{50} (LC_{90}) in % (w/v) causing 50(90)% inhibition of egg laying by females *T. urticae*, compared with untreated control

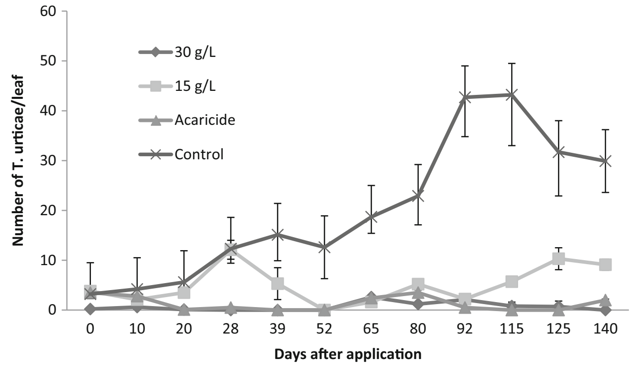

Fig. 3 Mean number of two-spotted spider mite (*T. urticae*) individuals on tomato leaves

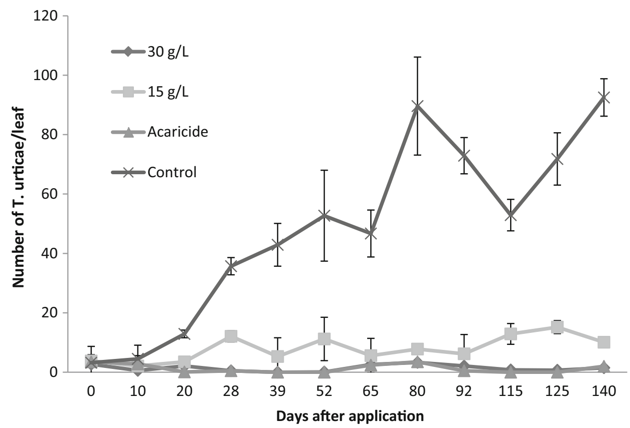

Fig. 4 Mean number of two-spotted spider mite (*T. urticae*) individuals on cucumber leaves

counts of cucumber and tomato fruits compared to the control (Table 2). At the same time, a positive effect of the extract was observed with respect to the mean weight of the fruits, where the weight was significantly higher not only compared to the control but, for the cucumbers, also compared to the applied acaricide. Overall yields of the tomatoes were significantly higher compared to both the positive and negative controls, where the mean weight of the fruits from one plant was 4.88 kg and 4.75 kg for extracts prepared from 30 and 15 g L^{-1}, respectively. A significant difference in the yields was also found for the cucumbers, although only compared to the untreated control, where the amount of fruits harvested from one plant was higher by more than 1 kg of fruits (Table 2).

Discussion

The search for new alternatives for fruit and vegetable protection is very important in terms of the production of safe foods. Recently, great efforts have been made in the search for new active substances of plant

Table 2 Average number and weight of fruits harvested on plants of cucumber and tomato

Dose	Tomatoes*			Cucumbers*		
	Number of fruits/plant (No ± SE)	Average weight/fruit (g ± SE)	Yield/plant (g ± SE)	Number of fruits/plant (No ± SE)	Average weight/fruit (g ± SE)	Yield/plant (g ± SE)
30 g/L	68.2 ± 1.1[a]	71.5 ± 0.6[a]	4878.8 ± 60.8[a]	16.7 ± 0.5[a]	361.1 ± 3.7[a]	6046.2 ± 176.8[a]
15 g/L	66.5 ± 0.6[ab]	71.5 ± 1.2[a]	4754.5 ± 91.8[a]	16.0 ± 0.4[a]	355.6 ± 6.7[ab]	5683.2 ± 191.9[a]
Acaricide	64.7 ± 4.1[ab]	64.7 ± 1.0[b]	4259.5 ± 143.3[b]	15.5 ± 0.3[ab]	345.8 ± 5.9[bc]	5357.7 ± 76.3[ab]
Control	57.2 ± 1.1[b]	65.1 ± 1.1[b]	3723.0 ± 114.5[c]	13.7 ± 0.7[b]	341.5 ± 5.6[c]	4693.1 ± 240.5[b]
ANOVA *F*, *P***	4.52; 0.028	13.84; 0.000	24.25; 0.008	6.24; 0.01	10.27; 0.001	10.03; 0.001

* Mean number and weight of fruits harvested on plants of cucumber and tomato (±S.E) within a column followed by the same letter do not differ significantly according to the least significant difference (Turkey's HSD test, $P < 0.05$)

** ANOVA parameters—*F*-value, *P*-significance level

origin that could become active substances of botanical insecticides (BIs) (Isman and Grieneisen 2014). However, complex, lengthy and financially costly authorisation processes have become a great hindrance for the practical implementation of achieved research results, i.e. in the production of commercial BIs (Isman 2015). The European Union has responded to the pressing need to speed up and simplify the process of introducing safe substitutes for synthetic pesticides in growing practices by establishing new legislation. This regulation includes the new term "basic substances"(BSs), which provides the option of European-wide authorisation of substances, of no concern with respect to any potential health risks, which are not primarily used for plant protection but which may be beneficial in the fight against harmful agents. Substances that are commonly used in the food industry or are otherwise consumed by people without any concerns are expected to be the main source of BSs (Pavela 2016b).

This study has therefore focused on the practical possibility of utilizing simply prepared extracts from the roots of *S. officinalis* as potential BSs, applicable as a substitute for the present acaricides against *T. urticae*. As found previously, extracts from this plant provide acaricidal effects (Pavela 2016a). However, no further information had been known before that time. In our study, we found that for acute toxicity, LC_{50} ranged between 0.3 and 1.2% (w/v) and LC_{90} between 1.2 and 1.7% (w/v), depending on the developmental stage (see Table 1). The greatest sensitivity was shown by eggs and nymphs and the least by adults. The extract also showed significant inhibition of oviposition by females on the treated plants. However, this phenomenon was observed in relatively high concentrations ($LC_{50} = 0.9\%$ and $LC_{90} = 3.8\%$). Based on these observations, we asked two basic questions: I. What amount of roots is needed to achieve the concentration of 1.5% (w/v), which caused higher than 90% mortality of the nymphs and eggs and more than 50% mortality of the adults; and II. For how long do we have to extract the roots so that a sufficient amount of active substances is released? As we found, approximately 30 g of the roots has to be extracted for at least 25 min in one litre of water to achieve the dissolution of at least 15.9 g of substances contained in the roots. As indicated by the tests, the roots contain approximately 50% of substances that are easily and rapidly extracted in water. Such a high percentage is given by the chemical composition of the roots, which contain a lot of quillaic acid and gypsogenin saponins (Lu et al. 2015). The roots were found to contain more than 30% of saponins (Yudina et al. 2007a). The dry mass of an extract obtained using organic solvents contains more than 89% of saponins (Frolova et al. 2013; Sadowska et al. 2014).

Saponins contained in the roots can be used for many current applications. Thanks to their chemical and physical properties, soapwort extracts have been used as emulsifiers and softening agents in the food industry, particularly in the production of "halva" and other sweets. Sunflower "halva" is a popular and widely enjoyed confectionery product specific to the countries of Eastern Europe (Bedigian 2004; Korkmaz and Özçelik 2011; Mureşan et al. 2013). In addition, extracts from *S. officinalis* have been traditionally used in medicine and in the cosmetics industry (CAS No. 84775-97-3) as diaphoretic, antioxidant and tonic agents (Kucukkurt et al. 2011). They have been traditionally used for the treatment of rheumatic diseases, syphilis and tetter, and for jaundice and engorgement of the abdominal viscera (Medeiros and de Albuquerque 2012). In addition, the effects of triterpene glycosides (saponins), recently extracted from *S. officinalis* radices, on the cellular and humoral innate immunity factors were studied (Kuznetsova et al. 2014). Tests showed a positive impact on natural immunity given that they stimulated the phagocytic, bactericidal and adhesion activities of polymorphonuclear leucocytes. The authors determined optimal conditions of saponin treatment for mice. Saponins promoted the maturation of human peripheral blood dendritic cells, which was proven by a high expression of the terminal differentiation marker and bone-stimulating molecule on the cell membrane. Moreover, no acute or chronic toxicity, or growth and tissue abnormalities, were found for the extract from the roots of *S. officinalis* administered orally to mice (Yudina et al. 2007b). It was also found that plants rich in triterpenoid saponins are a diet-dependent potential factor that has an important role in modulation of rumen fermentation processes (Szczechowiak et al. 2013). These findings provide evidence of the health safety of the extracts, including their potential residues, which may occur on vegetables treated using this extract.

We have verified the biological efficacy of the extracts in one-year greenhouse experiments in two vegetable species that are most commonly damaged by feeding of *T. urticae*. As we found, extracts obtained by maceration of the roots in a dose of 30 g L^{-1} resulted in a significant reduction in the incidence of this pest on the plants, and repeated application maintained this pest below the threshold of harmful effects. Moreover, substances contained in the extract had a positive effect on the number and weight of the fruits, which was manifested by an increased overall yield, compared to both the untreated control and also (for tomatoes) the standard treatment using a commercial acaricide based on a.i. abamectin. This increase may have been caused by the fertilizing effect of the substances contained in the extract, which may have been reflected in the higher weight of the fruits. In our tests, we used no fertilizers for application on the leaves or at the roots during the vegetation period. The soil was fertilized only before planting, using a basic dose of inorganic fertilizers. And although no lack of nutrients was observed, the extracts may also have served as a foliar fertilizer, with nutrients easily receivable by the plants. However, further experiments will be needed to confirm or disprove this hypothesis; in particular, the content of acceptable nutrients in the extract will have to be ascertained. Similarly, it will be important to determine the speed of degradation of active substances dissolved in water in order to derive a practical recommendation for growers regarding the possible storage period of the prepared extract. Currently, we propose using the extract within 24 h from extraction, which is the period verified by us in terms of efficacy of the extract.

The positive effect of the extract on the plant and fruit development of vegetables is yet another valuable property of extracts from the roots of *S. officinalis* that we found. Another positive property can be seen in the fact that the extract contains a mix of various saponins (Jia et al. 1998, 1999a, b; Lu et al. 2015) whose major contents enable us to expect that these are the substances responsible for the acaricidal efficacy, although their mechanism of action on mites is still waiting to be explained. A mixture of several substances with different expected mechanisms of action could significantly prevent the development of resistance in *T. urticae*; resistance (Isman 2006; Pavela 2014b) of this species against many commercial acaricides has been found, including products based on abamectin (Villegas-Elizalde et al. 2010).

Conclusion

To conclude, we can note that the extract obtained from 30 g of the roots of *S. officinalis* extracted for at least 25 min in one litre of water provides a significant reduction in the incidence of all developmental stages of the two-spotted spider mite in greenhouse-grown plants. The efficacy of repeated application was sufficient to maintain *T. urticae* below the threshold of harmful effects. Moreover, repeated application had a positive effect on the yields of tomato and cucumber fruits. Considering that the extract from the roots is primarily used in the food industry, traditional medicine and in the cosmetics industry, we can presume that use of the extract for the protection of vegetables against *T. urticae* is of no concern. The extract can thus be proposed as a candidate BS, which exhibits a high potential for being authorized in EU countries as a “basic substance”, and which may be beneficial for the growing of greenhouse vegetables. The utilization of extracts from the roots of *S. officinalis* as a substitute for existing acaricides can enable us to achieve a significant reduction in the risks related to application of synthetic pesticides, which will lead to increased “safety” of fresh vegetables such as cucumbers and tomatoes. However, successful introduction of *S. officinalis* extracts in practice will also depend on the costs associated with their application, which should be balanced by suitable subsidies offered to the growers.

Author’s contribution

RP conceived and designed research and his team conducted experiments. RP analysed data and wrote the manuscript. The tasks of preparation, application of the substances and evaluation of the experiments were performed by the technical team comprising IK, LS, IS and SM as named in the acknowledgements, who performed their work under the expert supervision of RP.

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