

EVALUATION OF SUSTAINABLE STRATEGIES FOR GREENHOUSE PEST CONTROL IN CHRYSANTHEMUM AND SWEET PEPPER PRODUCTION

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ABSTRACT

The effectiveness of 16 examples of selected sustainable practices in pest control (i.e. application of plant-derived products, microbial agents or inorganic compounds with expected low environmental impact; simultaneous use of already registered active substances against other harmful organisms, and trap cropping) was tested in the protected cultivation of *Chrysanthemum* × *morifolium* Creamist Golden and *Capsicum annuum* Ożarowska against the two-spotted spider mite (*Tetranychus urticae* Koch.) and thrips (*Frankliniella occidentalis* (Pergande), *Thrips tabaci* Lind.). The study identified *Bacillus subtilis*, common nettle manure, willow bark decoction, oregano and cinnamon essential oils as the most promising solutions for reducing spider mite population. However, in the thrips control, the infusion of Canadian goldenrod root showed a high immediate efficacy that was comparable to the abamectin that was used as a reference product. Further research on these substances is recommended to increase their effectiveness, understand their mode of action against pests and determine the impact on crops.

Keywords: two-spotted spider mite, *Tetranychus urticae*, thrips, protected cropping, natural insecticides, integrated pest management

INTRODUCTION

Recently, trends in horticultural production have aimed at minimising its negative impact on the environment, producing safe and residue-free plant products, as well as reducing or even eliminating the use of chemical pesticides. These endeavours are reflected in the growing emphasis on sustainable approaches to plant protection management, such as organic farming and the integrated pest management (IPM) [Baker et al. 2020, Deguine et al. 2021].

In this context, horticultural crops that are grown under cover (e.g. in greenhouses or polytunnels), due to being susceptible to strong pest pressure, have become an area of particular concern. High, stable tem-

peratures and periodically low air humidity levels, coupled with plants being grown as closely as possible to maximise the limited available space, intensify the incidence of common greenhouse pests. These include various species of aphids, thrips or spider mites [Perdikis et al. 2008, Fatnassi et al. 2015]. Due to the favourable microclimatic conditions, greenhouse pests are able to develop a larger population in a relatively shorter time and to complete more generations than in open field crops. This implies high pesticide use, but also the risk of pests developing resistance to currently authorised active ingredients [Tirello et al. 2012, Mani 2022].

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Excessive use of chemical plant protection products particularly affects protected fruit and vegetable crops, as they are usually consumed fresh [Allen et al. 2015, Mahdavi et al. 2022]. Nonetheless, there is a growing awareness of the potentially negative impact of pesticides, not only on employees in the ornamental horticulture sector, but also on the end consumers of ornamental plants, for example, through the residues of active ingredients in cut flowers [Toumi et al. 2016, Pereira et al. 2021]. Therefore, the need to develop alternative or complementary solutions in pest control is increasingly widely discussed in order to support the gradual shift away from dependence on chemical pesticides in greenhouse horticulture.

In order to identify promising strategies that could support sustainable pest control in protected crops, a search of academic databases (Web of Science, Google Scholar) was performed. The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) – hereafter referred to as TSSM – was selected as the target species due to its relevance as a widespread polyphagous pest of most protected crops [Perdikis et al. 2008]. The search covered publications up to April 2025 and used combinations of the following general keywords: *Tetranychus urticae*, *two-spotted spider mite*, *sustainable pest management*, *protected crops*, *greenhouse*, *natural compounds*, *botanical pesticides*. Moreover, a backward and forward snowballing approach was used to identify additional relevant studies from the reference lists of selected papers. As a result, the five general practices have been selected for testing: (i.) application of plant-derived products, e.g. extracts, decoctions, manures or essential oils [Kheradmand et al. 2015, Durán-Lara et al. 2020, Jakubowska et al. 2022]; (ii.) use of microbial control agents, including bacteria and fungi [Shinde et al. 2010, Al-Azzazy et al. 2020, Chouikhi et al. 2022]; (iii.) use of inorganic compounds, natural or manufactured, but with a low expected environmental impact [Alhewairini and Al-Azzazy 2018, Abdelwines and Ahmed 2024]; (iv.) exploiting the insecticidal or acaricidal side-effects of active ingredients already authorised for use against other harmful organisms, e.g. fungicides [Sukhoruchenko et al. 2021]; (v.) companion planting [van den Boom et al. 2003].

Sweet peppers and chrysanthemums were selected as test plants for assessing the effectiveness of the in-

vestigated pest management strategies. Both species hold a prominent position in the Polish greenhouse horticulture sector. For example, in 2023, the total area of crops cultivated under cover in the spring cycle in Poland was 4451.5 ha, with peppers being the leading crop, accounting for 39% of this area. The harvest of peppers in protected cultivation was estimated at 258.7 thousand t, second only to tomatoes [GUS 2024]. Chrysanthemums also play an important role in Polish ornamental plant production. Moreover, both species are suitable model plants for evaluating the effectiveness of spider mite control, with the additional advantage of being combined within a single greenhouse production cycle during the experiment (chrysanthemums following peppers).

The aim of the present study is to assess the effectiveness of various practices suitable for integrated and/or organic production systems in greenhouse pest control, using the example of the protected cultivation of sweet pepper and chrysanthemum. It was hypothesised that at least one of the practices tested would result in a significant reduction in the pest abundance compared to untreated control.

MATERIALS AND METHODS

The experiment was performed in a greenhouse complex belonging to the National Institute of Horticultural Research in Skierniewice, Poland (51°57'36.9"N, 20°08'59.5"E). Two separate, consecutive trials were conducted and the species tested were sweet peppers (*Capsicum annuum*) Ożarowska (from 24th May to 21st June 2024) and chrysanthemums (*Chrysanthemum × morifolium*) Creamist Golden (from 24th July to 19th August 2024). The experimental timeline is presented in Figure 1.

Preparation of plant material. The pepper seedlings were prepared in-house, whilst the rooted chrysanthemum cuttings in growing trays were obtained from an external supplier, the TURSCY Horticultural Farm based in Rzgów, Poland. During the initial growing stage, the young plants were kept in an isolated growing chamber to prevent pest infestation. Two foliar treatments with the fungicide Previcur Energy 840 SL (530 g L⁻¹ propamocarb + 310 g L⁻¹ fosetyl-Al) were performed; no other plant protection products were used. The plants were transplanted into

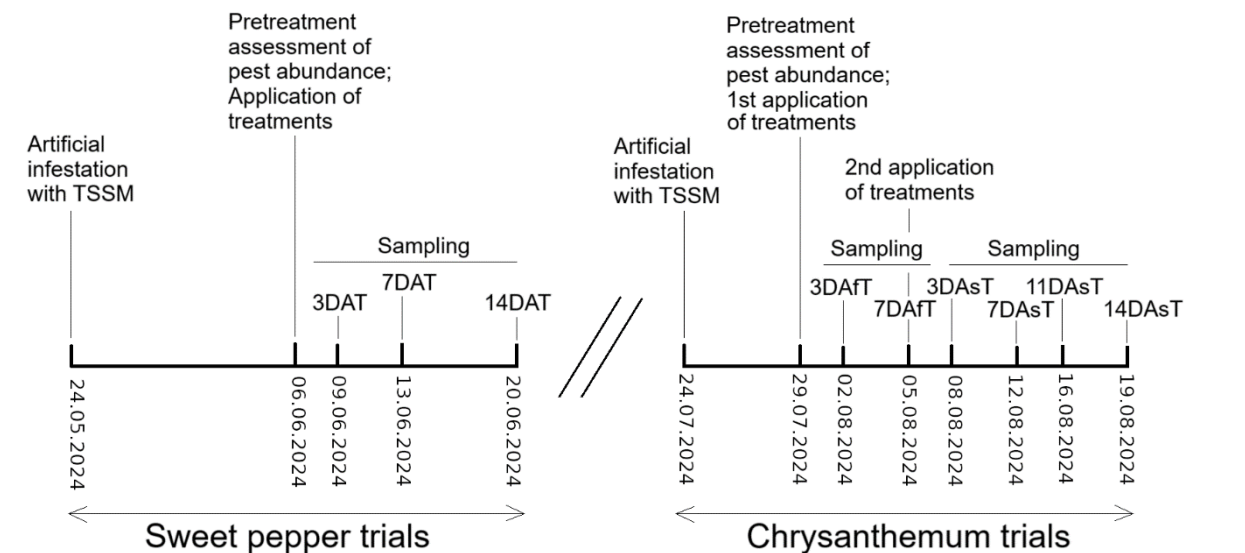


Fig. 1. Timeline of the greenhouse experiments conducted on sweet pepper (from 24th May to 21st June 2024) and chrysanthemum (from 24th July to 19th August 2024), showing the schedule of treatments and assessments. Explanations: TSSM – two-spotted spider mite; DAT – days after treatment; DAfT – days after the first treatment; DAiT – days after the second treatment

1.5 L plastic pots filled with peat-based horticultural medium (H2–H5; pH 6.0–6.5), thoroughly mixed with YaraMila Complex multinutrient fertiliser at a ratio of 1.5 g fertiliser to 1 L medium. Finally, the plants were moved to the target location of the experiment, where they were subjected to pest infestation: the pepper on 24th May 2024 at the stage where the first flower bud was visible (BBCH 51), and the chrysanthemum on 24th July 2024 at the stage before the formation of the flower bud (fewer than 20 true leaves on the main shoot).

Pest breeding and plant infestation. Male and female *T. urticae* mites were collected from an organic strawberry plantation (51°56'46.6"N, 20°11'11.2"E) and bred on common bean plants (*Phaseolus vulgaris*) cv. Eureka in an isolated greenhouse chamber, as described by Byrdy [1965]. Artificial infestation was performed by placing bean leaves with visible feeding symptoms (i.e. with a large but unidentified number of spider mites) on the crops, one leaf per each pepper or chrysanthemum plant. The leaves were not removed afterwards and were left to dry naturally. The incubation period lasted two weeks in the pepper trials and one week for the chrysanthemums, with the differenc-

es being due to the higher temperature in July, which favoured the pest's faster development.

In addition, during the pretreatment sampling stage, it was detected that a relatively abundant population of thrips spontaneously occurred in the pepper trials and the decision was taken to include this in the study. A random sample of 30 individuals was therefore collected by shaking them off the leaves and inflorescences. The microscopic preparations were made using the Heinz (PVA) medium. The western flower thrips (*Frankliniella occidentalis* (Pergande)) and the onion thrips (*Thrips tabaci* Lind.) were identified based on the work of Łabanowski [1992]. However, the spontaneous thrips population in the chrysanthemum trials was too scarce and uneven to enable statistical procedures, so the results were excluded.

Trial layout. The methodology was based on the standards of the European and Mediterranean Plant Protection Organisation (EPPO) No PP 1/37(2) and PP 1/160(2) – both modified for the purposes of the present experiment. For both species, each treatment was represented by 24 potted plants, arranged in groups of eight per compartment across three differ-

ent greenhouse chambers (for more information, see Fig. 2). The separate experimental chambers ($8 \times 4 \times 2.8$ m) were located in different parts of the greenhouse complex (one in the centre and two at opposite edges). During treatment applications, each plant was sprayed individually. During subsequent maintenance procedures and watering, the potted plants were repositioned within the compartments. Therefore, the assumption was made that each plant was an independent sampling unit, with the greenhouse chamber being regarded as a random effect. During the experiment, the microclimatic conditions were automatically recorded by a sensor system at 30-minute intervals, separately for each greenhouse chamber (Fig. 3).

A total of 16 different treatments were tested across the pepper and chrysanthemum trials, with 10 treatments included in each trial and some overlap between them (Tab. 1). The treatments were compared to water-treated control and abamectin as a reference product. Doses were applied according to the manufacturers' recommendations or, in the case of self-prepared preparations, based on previous laboratory tests (not presented). The tested preparations were dissolved (or suspended) in tap water. For each treatment, approx. 40 mL of liquid per plant were sprayed using a Kwarzar Orion manual pressure sprayer. The treatments were carried out late in the evening, at a temperature of approx. 23–24 °C. Careful attention was paid to covering



Fig. 2. The potted pepper and chrysanthemum plants were cultivated on greenhouse growing benches lined with capillary matting. Within a single greenhouse chamber, each treatment was represented by eight potted plants grouped in separate compartments measuring 1×1 m. To reduce the risk of pests moving between adjacent compartments, each was separated by an air-permeable fabric with a mesh size of 0.20×0.15 mm

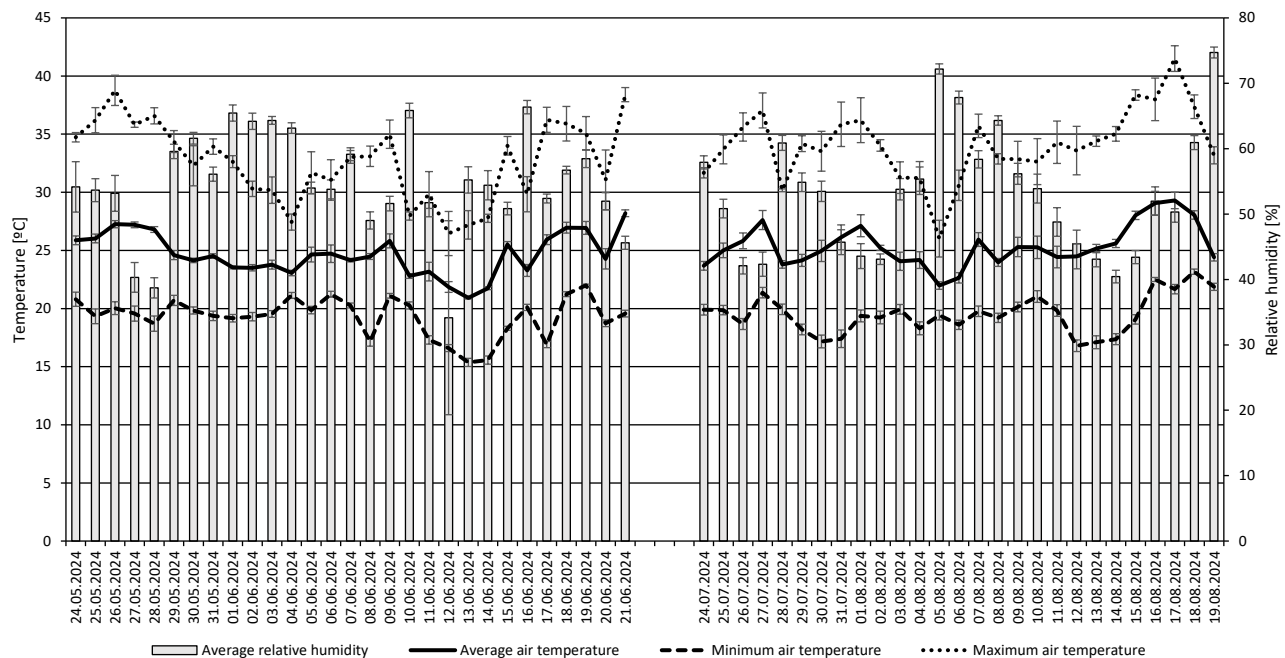


Fig. 3. Temperature and humidity conditions in the experimental greenhouse chambers in which the trials were re-peated. The period from 24.05.2024 to 21.06.2024 covers the experiments conducted on sweet peppers, while the period from 24.07.2024 to 19.08.2024 includes the experiments on chrysanthemums. The values presented are the daily arithmetic means of the particular parameters based on the recordings from all experimental chambers ($n = 3$), with the standard deviation included

the undersides of the leaves when spraying. Only one treatment was applied to the peppers (6th June 2024), whereas the chrysanthemums were sprayed twice – on 29th July 2024 and one week later. The only exception was the companion planting trial, in which the pepper plants were sprayed with water but placed together with soybeans, with four free-of-pest soybean plants per replicate, in such a way that their leaves were touching. The soybean plants were then removed after one week.

During the experiment, no fertilisers, biostimulants, growth regulators or pesticides (other than those foreseen for the particular treatments) were applied to either the pepper or the chrysanthemum trials. Soil water capacity was maintained at a steady level, with the pots being watered manually 2–3 times per week with approx. 50 mL of tap water. All side shoots were removed by hand as they appeared, leaving only one main shoot. No artificial light was used. The chrysanthemums were not covered with black-out material.

Sampling methodology and pest pressure assessments. At all sampling dates, samples of one leaf from each plant were collected randomly from the middle parts of the shoots. The live mites (including mobile forms – larvae and adults) and eggs, as well as thrips present on the leaves, were counted under the stereoscopic microscope. For both plant species, the initial sampling was performed immediately prior to the first treatment to assess the pretreatment pest pressure (in order to consult the presumptive differences resulting from the artificial TSSM infestation). Moreover, in the pepper trials, the post-treatment assessment was performed three times, at 3, 7 and 14 days after treatment (DAT), whilst there were six sampling dates in the chrysanthemum trials: 3 and 7 days after the first treatment (DAfT), and 3, 7, 11 and 14 days after the second treatment (DAsT).

To determine the size of the mite or thrips populations over time, the cumulative index of infestation

Table 1. Specification of treatments applied to control the greenhouse pests

Treatment(s)	Additional information, manufacturer/ method of preparation	Practice tested*	Dose (per 1 L of tap water)	Crop
Abamectin	Safran 018 SC; Rotam Agrochemical Europe Ltd.	Control (reference product)	0.5 mL	Sweet pepper, chrysanthemum
Willow bark decoction	50 g of dried <i>Salicis cortex</i> was boiled in 2700 mL of water for 30 minutes, then left to stand for 8 hours, and filtered	<i>i.</i>	100 mL	Sweet pepper, chrysanthemum
Infusion of Canadian goldenrod roots	200 g of wet <i>Solidago canadensis</i> rhizome was finely chopped, poured with 400 mL of water, then left to stand for 8 hours, and filtered	<i>i.</i>	100 mL	Sweet pepper
Tansy extract	Commercially available extract from <i>Tanacetum vulgare</i> ; BROS Ltd., Poznań, Poland	<i>i.</i>	50 mL	Chrysanthemum
Nettle and common wormwood manures	1000 g of wet herb of <i>Urtica dioica</i> or <i>Artemisia absinthium</i> was poured with 1 L of water and left to ferment in a covered enamelled pot. The content was stirred regularly and water was added as it evaporated	<i>i.</i>	100 mL	Sweet pepper
Cinnamon, carnation and oregano essential oils	Water-soluble oil dispersions were obtained from BCHEM Tomasz Miśkiewicz, Rusiec, Poland	<i>i.</i>	50 mL	Cinnamon oil – sweet pepper and chrysanthemum; oregano and carnation oils – chrysanthemum only
Biopuls Abracadabra	Commercially available microbial biostimulant containing <i>Beauveria</i> spp. and <i>Metarhizium</i> spp.; Microlife Ltd., Poznań, Poland	<i>ii.</i>	7 mL	Sweet pepper
Serenade ASO	Microbial fungicide containing <i>Bacillus subtilis</i> strain QST 713; Bayer AG, Leverkusen, Germany	<i>ii., iv.</i>	16 mL	Sweet pepper, chrysanthemum
<i>Akanthomyces lecanii</i> , <i>Penicillium</i> sp.	Entomopathogenic fungi isolated from horticultural soil; spore density of <i>A. lecanii</i> – 9.7×10^6 per mL, spore density of <i>Penicillium</i> sp. – 1.4×10^6 per mL	<i>ii.</i>	25 mL	Chrysanthemum
Calcium carbonite	Pure (min. 98%) laboratory CaCO ₃ ; WARCHEM Ltd., Zakręt, Poland	<i>iii.</i>	50 g	Sweet pepper
Bisteran	Commercially available biostimulant (420–600 g L ⁻¹ hydrogen peroxide stabilised with silver ions); DESIO HOLDING Ltd., Częstochowa, Poland	<i>iii.</i>	0.5 mL	Chrysanthemum
Luna Sensation 500 SC	Commercial fungicide (250 g L ⁻¹ fluopyram + 250 g L ⁻¹ trifloxystrobin); Bayer SAS, Lyon, France	<i>iv.</i>	1.6 mL	Sweet pepper, chrysanthemum
Companion planting	Soybean plants (<i>Glycine max</i>) cv. Erica	<i>v.</i>	Not applicable	Sweet pepper

* *i.* – application of plant-derived products; *ii.* – use of microbial control agents; *iii.* – use of inorganic compounds with a low expected environmental impact; *iv.* – exploiting the insecticidal or acaricidal side-effects of active ingredients already authorised for use against other harmful organisms; *v.* – companion planting

(CII) was calculated according to the formula proposed by Wratten et al. [1979]:

$$CII = \sum_{i=1}^{k-1} \left[\frac{t_n}{2} (x_n + x_{n+1}) \right]$$

Where: k is the number of assessments performed; x_n , x_{n+1} are the numbers of mobile mites (or thrips) on two subsequent sampling dates; and t_n is the number of days between the sampling dates.

In addition, the pepper leaves were collected at 14DAT (using the same procedure described above for the pest assessment), scanned at 1200 dpi and subjected to a graphical analysis in the *pliman* programme. The leaf health status, expressed as the ratio of red ('symptomatic') to green ('healthy') pixels, was computed on this basis [Olivoto 2022].

Statistical analysis. The logarithmic transformation $\log(x + 1)$ of the data obtained was used to ensure a normal distribution and reach variance homogeneity (except for the CII values, calculated for mobile TSSM on chrysanthemum leaves, which were normally distributed according to the Shapiro-Wilk test).

A one-way analysis of variance (ANOVA) was used to test the significance of differences in the mean values of the pepper leaf health status between treatments.

An analysis of covariance (ANCOVA) was performed to evaluate the effect of treatments on the number of mobile TSSM recorded at different post-treatment sampling points. The response variable was the number of mobile TSSM per leaf at a specific time point. The model included two covariates: the pretreatment number of mobile TSSM and the pretreatment number of TSSM eggs, whilst treatment and greenhouse chamber number were included as fixed factors. The analogous approach was used for the number of TSSM eggs per leaf, with the pretreatment numbers of mobile TSSM and TSSM eggs indicated as covariates in this model, as well as for the number of thrips per leaf, where the pretreatment number of thrips was considered a covariate. In addition, to evaluate the overall effect of the included factors on pest population dynamics, a multivariate analysis of covariance (MANCOVA) was performed with all

post-treatment sampling points considered simultaneously as response variables.

An analysis of covariance (ANCOVA) was also used to assess the effect of treatments on the CII values calculated for mobile TSSM and thrips. In these models, treatment and greenhouse chamber number were included as fixed factors, whilst pretreatment counts served as covariates: the pretreatment numbers of mobile TSSM and TSSM eggs were used for the CII of mobile TSSM, and the pretreatment number of thrips was included for the CII of thrips. For these analyses, mean values aggregated at the greenhouse chamber level were used.

Normality (assessed using the Shapiro–Wilk test) and homogeneity of variance (Levene's test) of residuals were confirmed for all the models. The Newman-Keuls test was applied as a post hoc procedure to compare the differences between means. The significance level of $\alpha \leq 0.05$ was adopted for testing the hypotheses. The STATISTICA v.13.3 package was used for all the statistical calculations.

Data availability. The raw experimental data that support the findings of this study are publicly available in the RepOD Repository for Open Data at <https://doi.org/10.18150/2WGHRP>, reference number 2WGHRP.

RESULTS

Direct effects on the crops. Throughout the observation period, no visible phytotoxic effects (e.g. no chlorosis, stunting, leaf discolouration, deformations, etc.) in response to the pest control methods tested were recorded, in the case of sweet pepper or chrysanthemum plants. A white residue appeared on the pepper leaves and stems after they were sprayed with an aqueous solution of calcium carbonate, however this could be easily wiped off and did not cause any permanent damage.

The above general observations are reflected in the results of the graphical analysis of pepper leaves. The leaf health status did not depend on the type of treatment ($F_{11,251} = 0.90$, $p = .54$, $\eta_p^2 = 0.04$), with the average share of symptomatic leaf area varying slightly between treatments in the range of 0.505–0.588% (detailed results are not presented).

Mobile TSSM on pepper leaves. The mean number of mobile TSSM on the pepper leaves depended significantly on the type of treatment (Wilks' $\lambda = 0.45$, $F_{33,796} = 7.52$, $p < .001$, $\eta_p^2 = 0.24$) and the differing conditions between the greenhouse chambers (Wilks' $\lambda = 0.55$, $F_{6,540} = 31.54$, $p < .001$, $\eta_p^2 = 0.26$). The pretreatment numbers of mobile TSSM (Wilks' $\lambda = 0.98$, $F_{3,270} = 1.51$, $p = .21$, $\eta_p^2 = 0.02$) and TSSM eggs (Wilks' $\lambda = 0.99$, $F_{3,270} = 0.64$, $p = .59$, $\eta_p^2 = 0.01$) explained the variability of this parameter to a negligible extent, with no statistically significant effect.

Common wormwood manure and Serenade ASO significantly reduced the number of spider mites compared to the control at 3DAT, achieving similar efficacy to abamectin (Fig. 4). However, one and two weeks after treatment, only the abamectin application showed a significantly lower number of mobile TSSM

compared to the control. The neighbouring soybean plants tended to increase the spider mite pressure on the sweet peppers at all of the sampling dates, although this effect was not confirmed by the statistical test (Fig. 4). Meanwhile, the soybean leaves were severely infested by the pest (an average of 363.0 ± 227.4 mobile TSSM per trifoliate leaf on day 10 after the start of the experiment, $n = 32$).

The CII calculated for mobile TSSM depended significantly on both the treatment type ($F_{11,20} = 2.41$, $p = .04$, $\eta_p^2 = 0.57$) and the number of the experimental chamber ($F_{2,20} = 12.39$, $p < .001$, $\eta_p^2 = 0.55$), with no significant impact of pretreatment numbers of mobile TSSM ($F_{1,20} = 0.07$, $p = .79$, $\eta_p^2 = 0.00$) or TSSM eggs ($F_{1,20} = 0.06$, $p = .81$, $\eta_p^2 = 0.00$). A comparison of the CII values (Tab. 2) shows that only abamectin significantly reduced the spider mite pressure on the pep-

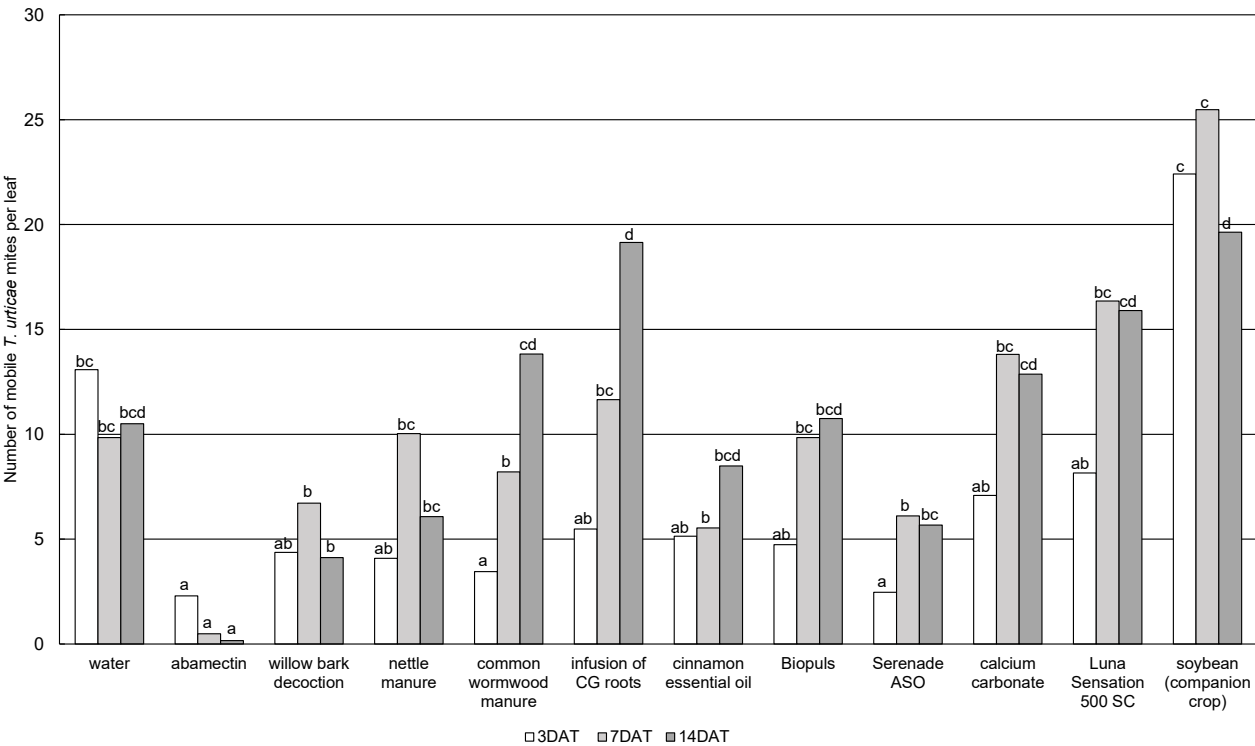


Fig. 4. Effects of different pest control methods on the number of mobile (larvae and adults) two-spotted spider mites on sweet pepper leaves at 3, 7 and 14DAT (days after treatment). Bars represent $(\text{antilog } x) - 1$ detransformed means ($n = 24$). Different letters above the bars within each sampling date indicate significant differences at $\alpha = 0.05$, according to the Newman-Keuls test. Explanation: CG – Canadian goldenrod

Table 2. Effects of pest control measures applied on the cumulative index of infestation (CII), calculated for mobile (larvae and adults) two-spotted spider mites on sweet pepper leaves throughout the entire observation period (3–14 days after treatment). The values presented are $(antilog x) - 1$ detransformed means ($n = 3$)

Treatment	Cumulative index of infestation
Water	1933.3 bc*
Abamectin	111.5 a
Willow bark decoction	997.9 b
Nettle manure	1202.0 b
Common wormwood manure	1589.1 bc
Infusion of Canadian goldenrod roots	2226.8 c
Cinnamon essential oil	1038.3 b
Biopuls	1914.7 bc
Serenade ASO	1053.2 b
Calcium carbonate	1913.5 bc
Luna Sensation 500 SC	2235.1 c
Soybean (companion crop)	3583.3 d

* Means marked with different letter(s) in the column are significantly different at $\alpha = 0.05$, according to the Newman-Keuls test

per leaves, reaching approx. 94% efficacy compared to the control. None of the treatments tested showed efficacy comparable to abamectin over time. An intermediate result in the range of 37.8–48.4% efficacy was obtained after applying willow bark decoction, nettle manure, cinnamon essential oil and Serenade ASO. However, soybean as a companion crop almost doubled the infestation of spider mites in the pepper plants.

TSSM eggs on pepper leaves. The analysis revealed the significant effects of the type of treatment (Wilks' $\lambda = 0.66$, $F_{33,796} = 3.71$, $p < .001$, $\eta_p^2 = 0.13$) and the experimental chamber (Wilks' $\lambda = 0.55$, $F_{6,540} = 31.55$, $p < .001$, $\eta_p^2 = 0.26$) on the number of TSSM eggs on the pepper leaves. The effects of pretreatment numbers of mobile TSSM (Wilks' $\lambda = 0.98$, $F_{3,270} = 1.48$, $p = .22$, $\eta_p^2 = 0.02$) and TSSM eggs (Wilks' $\lambda = 1.00$, $F_{3,270} = 0.19$, $p = .90$, $\eta_p^2 = 0.00$) was not statistically significant.

Although an immediate (3DAT), statistically significant reduction in the abundance of pest eggs was recorded only if calcium carbonate was applied,

a downward trend was also observed in plants treated with abamectin, common wormwood manure, infusion of Canadian goldenrod roots, cinnamon essential oil, Biopuls, Serenade ASO and Luna Sensation 500 SC (Fig. 5). One week after treatment, fewer TSSM eggs were counted only on pepper leaves sprayed with abamectin. Nevertheless, a statistically non-significant decrease in response to the application of willow bark decoction, common wormwood manure and cinnamon essential oil should also be noted. Two weeks after treatment, a significant reduction in pest reproduction was observed in both the abamectin and willow bark treatments. However, as with the mobile forms of the pest, the neighbouring soybean plants seemed to favour the mite reproduction at all sampling dates, although this was not reflected in the statistical test results (Fig. 5).

Thrips on pepper leaves. The number of thrips on the pepper leaves depended significantly on both the type of treatment (Wilks' $\lambda = 0.69$, $F_{33,799} = 3.28$, $p < 0.001$, $\eta_p^2 = 0.12$) and the experimental chamber (Wilks' $\lambda = 0.88$, $F_{6,542} = 6.22$, $p < .001$, $\eta_p^2 = 0.06$).

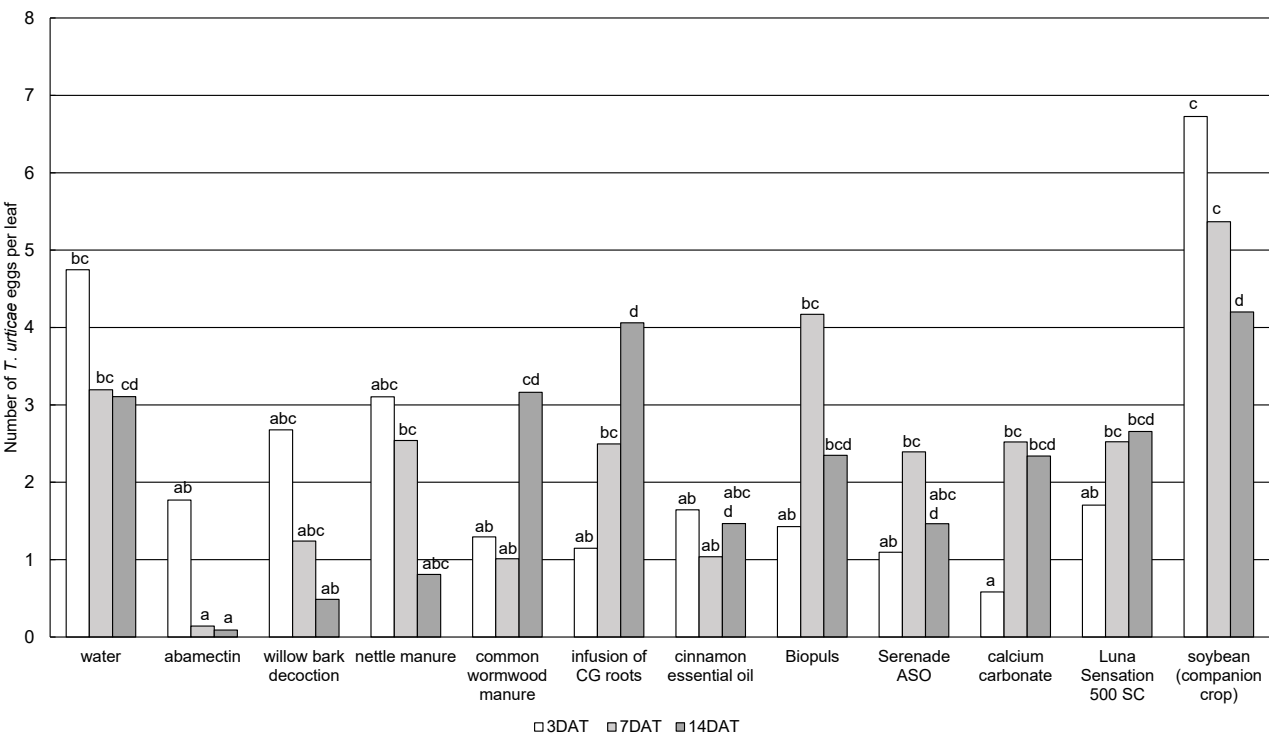


Fig. 5. Effects of different pest control methods on the number of two-spotted spider mite eggs on sweet pepper leaves at 3, 7 and 14DAT (days after treatment). Bars represent ($\text{antilog } x$) – 1 detransformed means ($n = 24$). Different letters above the bars within each sampling date indicate significant differences at $\alpha = 0.05$, according to the Newman-Keuls test. Explanation: CG – Canadian goldenrod

The impact of the pretreatment number of thrips (Wilks' $\lambda = 0.97$, $F_{3,271} = 2.94$, $p = .03$, $\eta_p^2 = 0.03$), although statistically significant, contributed only marginally to the variability of the results. On the other hand, neither the type of treatment ($F_{11,21} = 1.70$, $p = .14$, $\eta_p^2 = 0.47$) nor the experimental chamber ($F_{2,21} = 2.07$, $p = .15$, $\eta_p^2 = 0.17$) significantly affected the CII values computed for thrips (results not presented). This parameter significantly depended only on the pretreatment number of thrips on pepper leaves ($F_{1,21} = 12.02$, $p < .005$, $\eta_p^2 = 0.36$).

As shown in Figure 6, abamectin and infusion of Canadian goldenrod roots reduced significantly the number of thrips on pepper leaves shortly after being applied (3DAT). One week after treatment, only the abamectin-treated plants had fewer pests present, whilst none of the measures provided satisfactory thrips control one week later.

Mobile TSSM on chrysanthemum leaves. The type of treatment (Wilks' $\lambda = 0.43$, $F_{66,1434} = 3.74$, $p < .001$, $\eta_p^2 = 0.15$), the experimental chamber (Wilks' $\lambda = .82$, $F_{12,534} = 4.77$, $p < .001$, $\eta_p^2 = 0.10$), as well as the pretreatment population of mobile mites (Wilks' $\lambda = 0.95$, $F_{6,267} = 2.46$, $p = .02$, $\eta_p^2 = 0.05$) had a significant impact on the number of mobile TSSM on the chrysanthemum leaves although the extent of explained variability was inconsistent.

Significant differences between the control and the substances tested were not observed until 7DAfT, when fewer mobile TSSM were found on the abamectin-treated plants compared to those in the control (Fig. 7). An intermediate result of mite control was recorded after the application of willow bark decoction, tansy extract, oregano essential oil, *Penicillium* sp., Serenade ASO and Bisteran.

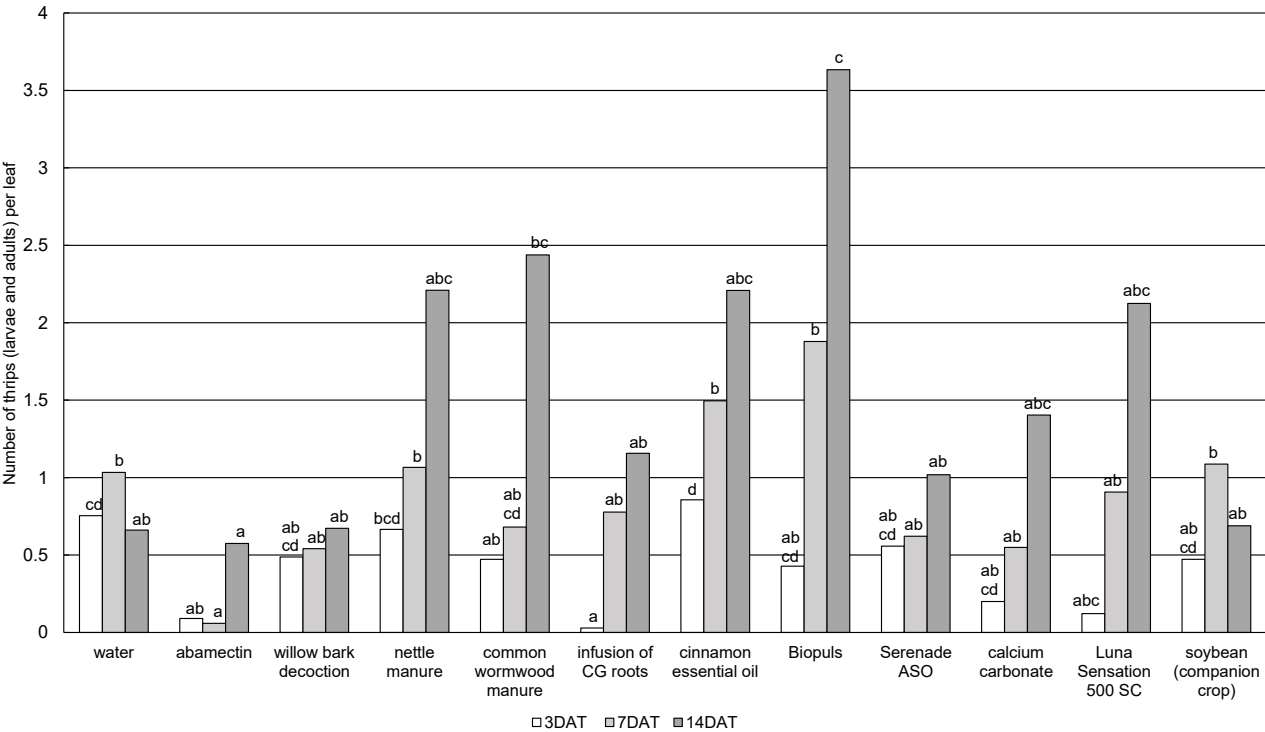


Fig. 6. Effects of different pest control methods on the number of thrips (larvae and adults) on sweet pepper leaves at 3, 7 and 14DAT (days after treatment). Bars represent ($\text{antilog } x$) – 1 detransformed means ($n = 24$). Different letters above the bars within each sampling date indicate significant differences at $\alpha = 0.05$, according to the Newman-Keuls test. Explanation: CG – Canadian goldenrod

In the second series of treatments, the positive effect of the substances tested was solely recorded three days after spraying. At this sampling date, abamectin significantly reduced the number of mobile TSSM compared to the control, whilst the carnation essential oil showed an intermediate efficacy. Later, between 7 and 14DAT, none of the control methods tested reduced the spider mite pressure on the chrysanthemums; in fact, there was a significant increase in the pest population on plants treated with Luna Sensation 500 SC compared to the control (Fig. 7).

The CII calculated for mobile TSSM present on the chrysanthemum leaves over all sampling dates enables a comparison of the overall response of the pest population to the treatments applied (Tab. 3). The values of this parameter depended significantly on the type of treatment ($F_{11,20} = 2.51, p = .04, \eta_p^2 = 0.58$), but not on the experimental chamber ($F_{2,20} = 2.99, p = .07,$

$\eta_p^2 = 0.23$), the pretreatment number of mobile TSSM ($F_{1,20} = 1.87, p = .19, \eta_p^2 = 0.09$) nor TSSM eggs ($F_{1,20} = 1.34, p = .26, \eta_p^2 = 0.06$).

According to the data presented in Table 3, abamectin showed the most promising trend towards reducing the pest population compared to the control, with oregano essential oil and Serenade ASO performing less well. Nevertheless, these results were not confirmed by significant differences in the statistical test.

TSSM eggs on chrysanthemums. Although the number of TSSM eggs depended on the type of treatment (Wilks' $\lambda = 0.53, F_{66,1134} = 2.71, p < .001, \eta_p^2 = 0.11$), significant differences between the treatments in the post-hoc comparisons only became apparent at certain time points and generally did not favour any of the approaches (Fig. 8). Other factors, i.e. the experimental chamber (Wilks' $\lambda = 0.88, F_{12,534} = 3.01, p < .001, \eta_p^2 = 0.06$), pretreatment numbers of eggs (Wilks' $\lambda = 0.99,$

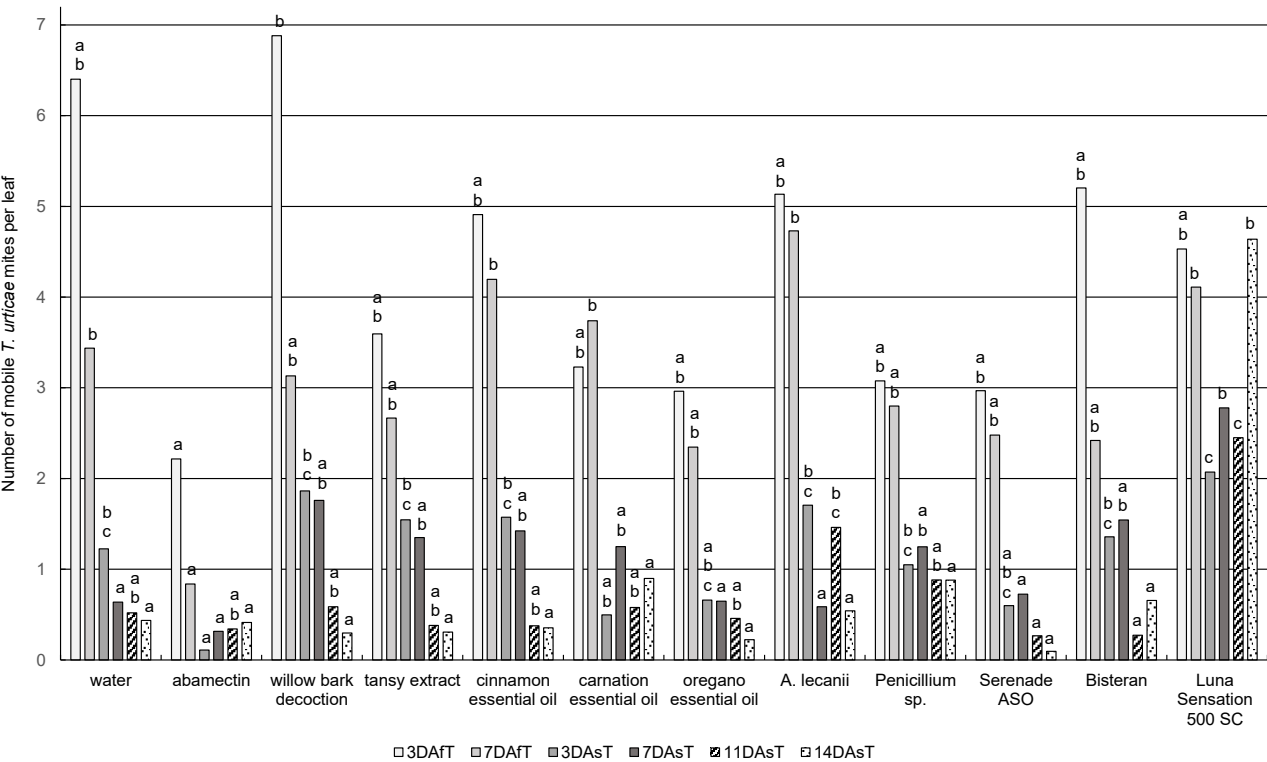


Fig. 7. Effects of different pest control methods on the number of mobile (larvae and adults) two-spotted spider mites on chrysanthemum leaves at 3, 7 DAfT (days after the first treatment) and 3, 7, 11, 14 DAsT (days after the second treatment). Bars represent $(\text{antilog } x) - 1$ detransformed means ($n = 24$). Different letters above the bars within each sampling date indicate significant differences at $\alpha = 0.05$, according to the Newman-Keuls test

$F_{6,267} = 0.58, p = .75, \eta_p^2 = 0.01$) and mobile forms (Wilks' $\lambda = 0.99, F_{6,267} = 0.68, p = .67, \eta_p^2 = 0.01$) of TSSM, had even lower or no explanatory power.

DISCUSSION

Sustainable horticultural production is significantly hampered by the high pressure of pests such as TSSM or thrips in protected cultivation. Therefore, there has recently been a strong research focus on the development of alternative pest control methods that could reduce the use of chemical pesticides in greenhouse horticulture. However, it is worth noting that the efforts of researchers have been quite fragmented, with many findings being overlooked and others remaining stuck at the laboratory stage, rather than serving as a stepping stone to future semi-field or full-scale trials

[van den Boom et al. 2003, Kheradmand et al. 2015, Abdelwines and Ahmed 2024]. In this context, the present study was intended as a means of collecting and testing various promising ideas with a view to outlining further directions for research on a larger scale, and it has fulfilled its objective.

Application of plant-derived products. This study assessed various plant-derived preparations with expected or previously reported pest control applications. Here, the sole focus will be on those which the results have demonstrated as being potentially useful in plant protection.

Of the essential oils tested, in the pepper trials, cinnamon oil performed relatively well against TSSM (almost 50% reduction in plant infestation) but not against thrips. In the chrysanthemum trials, on the other hand, cinnamon oil was not as effective. Better re-

Table 3. Effect of pest control measures applied on the cumulative index of infestation (CII) calculated for mobile (larvae and adults) two-spotted spider mites on chrysanthemum leaves throughout the entire observation period (3–7 days after the first treatment and 3–14 days after the second treatment). The values presented are arithmetic means \pm standard deviation (n = 3)

Treatment	Cumulative index of infestation
Water	444.7 \pm 96.8 ab*
Abamectin	149.5 \pm 70.9 a
Willow bark decoction	550.2 \pm 402.9 ab
Tansy extract	451.7 \pm 299.8 ab
Cinnamon essential oil	548.8 \pm 306.8 ab
Carnation essential oil	380.5 \pm 59.3 ab
Oregano essential oil	327.7 \pm 170.3 a
<i>A. Lecanii</i>	571.8 \pm 164.1 ab
<i>Penicillium</i> sp.	466.2 \pm 310.7 ab
Serenade ASO	317.0 \pm 270.1 a
Bisteran	459.2 \pm 213.7 ab
Luna Sensation 500 SC	791.0 \pm 176.1 b

* Means marked with different letter(s) in the column are significantly different at $\alpha = 0.05$ according to the Newman-Keuls test

sults were obtained by treating the plants with oregano oil, instead. Previous reports on the acaricidal activity of cinnamon oil include successful laboratory tests performed on the carmine spider mite (*Tetranychus cinnabarinus*) [Nasr et al. 2019], and of oregano oil on *T. urticae* [Tabet et al. 2018].

The usefulness of willow bark decoction for pest control is suggested by the approximate halving of the TSSM infestation level in peppers with, a similar downward trend observed for thrips. However, this effect was not observed in the chrysanthemum trials, which could be explained by differences in the response of particular plant species to the use of this substance and/or variation in microclimatic conditions (Fig. 3). Willow bark is currently approved under the EU regulations as a basic substance with fungicidal properties [Deniau et al. 2019], but no information exists on its application in pest control. It has been known that willow bark is a raw material rich in bioactive compounds, in particular phenolic compounds [Piątczak et al. 2020]. The pest control effect observed in this study could have been due to both the direct in-

teractions of the bioactive compounds with arthropods and the induction of natural plant defence processes [Favaro et al. 2019].

Furthermore, the results obtained in this study indicate a certain potential of nettle manure in the TSSM control. According to Dąbrowski and Seredyńska [2007], aqueous nettle extract not only affected the behaviour of *T. urticae*, repelling the pest and exhibiting antifeedant activity, but also caused direct mortality. However, in the current study, the liquid nettle manure was applied, and the different method of processing the herbal raw material (fermentation instead of extraction) may have resulted in a different chemical composition of the final product, also due to metabolites secreted by microorganisms. Thus, determining the chemical profile and microbial content of the liquid manure should be the starting point for further research.

A particularly interesting finding of this work is the high immediate activity of the Canadian goldenrod root infusion in thrips control, comparable to abamectin as a reference product. Canadian goldenrod

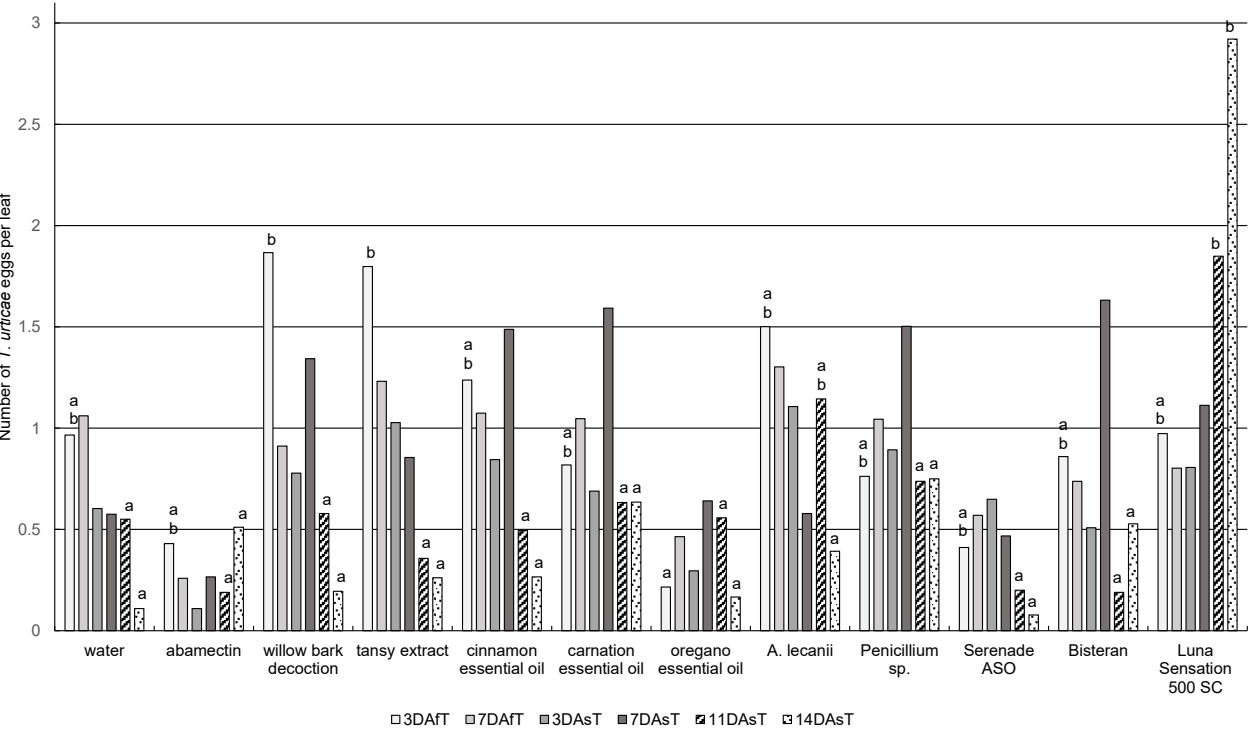


Fig. 8. Effects of different pest control methods on the number of two-spotted spider mite eggs on chrysanthemum leaves at 3, 7 DAFT (days after the first treatment) and 3, 7, 11, 14 DAsT (days after the second treatment). Bars represent $(\text{antilog } x) - 1$ detransformed means ($n = 24$). Different letters above the bars (if present) indicate significant differences at $\alpha = 0.05$ within each sampling date, according to the Newman-Keuls test

is an invasive, fast-spreading plant species in many Eurasian countries. It is difficult to eradicate due to its extensive rhizome system, from which the plants can grow back after the above-ground parts have been damaged [Królak 2021]. To date, only one study has been conducted on the potential use of this species in pest control, which identified the moderately repellent properties of the essential oils extracted from the leaves, stems and inflorescences of the Canadian goldenrod [Baranová et al. 2023]. Here, the idea was to evaluate the roots as a potential material in plant protection, assuming that positive results would contribute to the eradication of this alien species by harvesting (and thus destroying) its rhizomes from natural sites. However, the allelopathic effects of Canadian goldenrod on crops should also be taken into account [Możdżeń et al. 2020]. Further research should therefore focus on assessing the physiological response of

crops to the doses of goldenrod preparations used in relation to pest control efficacy, and on possible means of mitigating the negative impact.

Use of microbial control agents. Of the microbial control agents tested in this study, bacterium *B. subtilis* appears to be the most promising solution. Although the performance of the product containing it, Serenade ASO, was less pronounced compared to abamectin as the reference substance, the reduction in the TSSM infestation by almost 50% in pepper trials and 30% in chrysanthemum trials compared to water-treated control should be noted. Other studies also provide evidence of the acaricidal activity of *B. subtilis*, both in laboratory and field trials [Al-Azzazy et al. 2020, Emam 2021, Çelik et al. 2023].

Even though the mode of action of *B. subtilis* against phytophagous mites has not yet been sufficiently explained, Al-Azzazy et al. [2020] suggested

a possible direct bacterium-to-pest infestation, based on disease symptoms occurring in spider mites exposed to *B. subtilis*. An interesting research direction could also be to evaluate the impacts of various substances and toxins secreted by *B. subtilis* on spider mites, as well as to compare the control effectiveness between different bacterial strains [Nagórska et al. 2007]. In this study, *B. subtilis* strain QST 713 was applied in order to test its potential impact on pests as a currently registered biological fungicide. However, it is possible that other strains would have achieved a better result.

The other microbiological agents did not achieve the expected effectiveness in pest control, but the results of this study should not be considered definitive in this regard. The lack of effectiveness could be due to unfavourable microclimatic conditions in the experimental greenhouses (Fig. 3), as adequate humidity and temperature play a crucial role in the activity of entomopathogenic microorganisms [Shipp et al. 2003].

Use of inorganic compounds. Simple inorganic compounds could potentially offer an alternative to synthetic pesticides, whose residues are often detected in crops and cause concern for consumers. The chemical properties of calcium carbonate or hydrogen peroxide suggest they act through direct contact with a pest, e.g. by damaging the cuticle and/or causing water loss, optionally by repelling harmful arthropods. Additional benefits of using these substances may include, for calcium carbonate, increasing the tolerance of plants to heat stress and improving the fruit quality [Patanè et al. 2018, Ramírez-Godoy et al. 2018], while hydrogen peroxide (especially enriched with silver ions) might provide a biostimulant effect and plant disease prevention [Orlikowski et al. 2023].

However, the experiment carried out on peppers within this study, apart from the short-term effect of a decrease in TSSM reproduction soon after treatment, does not confirm the effectiveness of calcium carbonate in mite control, contradicting the findings of Abdelwines and Ahmed [2024]. Also, two treatments with Bisteran (which contains silver-stabilised hydrogen peroxide; it is the current equivalent of the product HuwaSan TR50 on the Polish market, which is approved as biostimulant for spraying directly on plants) proved ineffective in reducing the TSSM population on chrysanthemums. The unsatisfactory

efficacy of Bisteran could be explained by its insufficient concentration in the treatments performed. Alhewairini and Al-Azzazy [2018] documented a clear increase in the acaricidal activity of HuwaSan TR50 with increasing concentrations in the range of 0.1–0.4%. In this study, the spray was applied at the concentration permitted on the product label for ornamental plants (0.05%), which is probably too low to control the pest. Therefore, biostimulant treatments with Bisteran at the currently recommended dosage may not be sufficient to ensure a parallel pest control effect.

Insecticidal or acaricidal side-effects of already authorised active ingredients. A promising practice, especially in view of the decreasing availability of registered active substances, could be to plan plant protection treatments in such a way as to exploit the possible insecticidal or acaricidal side-effects of chemical products applied against other non-target harmful organisms. Such a possibility was tested by Sukhoruchenko et al. [2021], who reported the high (almost 100%), albeit delayed up to approx. two weeks after application efficacy of the fungicide Luna Tranquility (125 g L⁻¹ fluopyram + 375 g L⁻¹ pyrimethanil) in the reduction of the TSSM population. Furthermore, the same study showed the product's satisfactory aphicidal activity on the example of the peach-potato aphid (*Myzus persicae*), and the limited effectiveness in the control of the greenhouse whitefly (*Trialeurodes vaporariorum*). In conclusion, the authors suggested that the toxic effect of the fungicide on the non-target arthropods is related to the activity of fluopyram.

Indeed, according to the FRAC classification, fluopyram belongs to the group of SDHI fungicides (succinate dehydrogenase inhibitors). This enzyme plays an important role in both the citric acid cycle and oxidative phosphorylation. Its dysfunction impairs the process of cellular respiration in mitochondria [Bouillaud 2023]. In addition to its antifungal activity, fluopyram has also been found to act as an effective nematocide (classified to the group of mitochondrial complex II electron transport inhibitors – METI). However, it has been proven not to possess activity against other animals, including insects, oligochaetes and vertebrates, which is explained by amino acid differences compared to nematodes

within SDH complex, known to be important for interaction of fluopyram with the target [Schleker et al. 2022].

In the present study, the effect of another fungicide from the Luna series, Luna Sensation 500 SC was evaluated. The results obtained do not support the hypothesis of either the insecticidal or acaricidal activity of fluopyram. On the contrary, in the chrysanthemum trials, a significant increase in the number of both mobile TSSM and their eggs was found after the second application of the pesticide. The mechanism underlying this observation requires further investigation, as little is yet known about the layered interactions between fungicides and plant pests [Margus et al. 2023].

Companion planting. The dispersal of TSSM depends on its varying preferences for various host plants. In turn, host plants affect its reproductive potential and population growth rate [Greco et al. 2006]. In a study by van den Boom et al. [2003], contrasting differences in acceptance rate were observed between soybean and sweet pepper, with the pest showing a strong preference for the former. It was also noted that, given the opportunity, TSSM migrated from the pepper plants in search of an alternative feeding source.

Considering the growing popularity of trap cropping as a specific form of companion planting [Sarkar et al. 2018], as well as the findings outlined above, this study aimed to assess the development of the TSSM population on pepper plants in the simulated companion planting system with soybean. It was assumed that, once it had infested the peppers, the pest would migrate to the more attractive soybean plants, thus causing less damage to the cash crop. The removal of the soybean plants was intended to simulate potential agronomic operations, such as removing potted trap plants from greenhouses after the expected period of their infestation, or mowing, raking and removing the trap intercrop grown alongside.

Indeed, the pest migrated from the peppers to the expected trap plants and established a far more numerous population; this confirms that soybeans create a more favourable environment for TSSM. However, despite the removal of the trap plants, this did not prevent TSSM from infesting the peppers and increasing its pressure almost twofold, making the assessed practice counterproductive. No such stimulating effect

of soybean on the infestation of peppers was still observed in the case of thrips.

CONCLUSIONS

A screening study of several sustainable methods of greenhouse pest control allowed for the selection of the most promising solutions recommended for further research, i.e. microorganisms (*Bacillus subtilis*) and plant-derived products (willow bark decoction, oregano and cinnamon essential oils, common nettle manure, infusion of Canadian goldenrod roots). Future studies should focus on explaining their modes of action, increasing their effectiveness (e.g. by identifying synergists, developing strategies for combined and sequential application, extending the durability in the growing environment) and assessing their impacts on plants at the physiological level.

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