

EFFECT OF AQUEOUS EXTRACTS OF THREE DIFFERENT PLANTS ON MYZUS PERSICAE SULZER (HEMIPTERA: APHIDIDAE) INFESTING PEPPER PLANTS UNDER LABORATORY CONDITIONS

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ABSTRACT

This study aimed to determine the percentage infestation and population parameters of the green peach aphid *Myzus persicae* under laboratory conditions and evaluate the effect of aqueous extracts of three different plants (pot marigold: *Calendula officinalis*, mint: *Mentha viridis* and rosemary: *Salvia rosmarinus*) on the mortality of this aphid. Extracts of these plants were used at three concentrations (C1 = 15%, C2 = 30% and C3 = 45%). Results indicate a percentage infestation of 76.9 ± 9.4%, a mean relative growth rate of 0.062 ± 0.007 and generation time of 11.12 ± 1.42 days. All treatments reduced the numbers of aphids and statistically significantly reduced the number ($\alpha < 0.01$) recorded after treatment with C1, C2 and C3 of each extract. *C. officinalis* extract was more effective than those of *M. viridis* and *S. rosmarinus*. The highest mortality (69.82 ± 5.23%) and efficacy (61.71 ± 4.46%) were recorded for the C3 of aqueous extract of *C. officinalis*, whereas the lowest mortality (38.24 ± 2.42%) and efficacy (32.41 ± 1.23%) were recorded for the C1 of extract of *M. viridis*. The data provided indicate that aqueous extracts of *C. officinalis*, *M. viridis* and *S. rosmarinus* have an insecticidal effect on *M. persicae* and can be integrated into a pest management strategy to reduce *M. persicae* abundance on pepper plants.

Keywords: botanical extracts; efficacy; mortality; *Myzus persicae*; population parameters

Introduction

Pepper (*Capsicum* spp.) is an annual herbaceous plant belonging to the Solanaceae family, which includes commercial vegetables such as tomato, eggplant, tobacco and potato (Tripodi and Komar 2019). It is one of the most popular vegetable crops throughout the world and a source of vitamins C and E, provitamin A, carotenoids and phenolic compounds that offer health benefits for consumers (Agwu et al. 2018). Despite the importance and popularity of pepper, several pests, such as insects, fungi, bacteria, viruses and nematodes adversely affect its production worldwide.

Among the insects, the green peach aphid, *Myzus persicae* Sulzer (Hemiptera, Aphididae) is one of its most serious pests (Blackman and Eastop 2006; van Emden and Harrington 2007; Mdellel et al. 2019). It can occur on the underside of the leaves and on young plants at high densities, which causes deformation of leaves, reduces plant growth and infects the plants with virus, which reduce yield and the quality in terms of its nutritional content (Blackman and Eastop 2006; Bass et al. 2014). The green peach aphid is controlled mainly by using chemical insecticides.

However, the use of chemical insecticides has serious disadvantages, such as human and animal poisoning, water contamination, development of pest resistance, death of pollinating agents and natural enemies (Ofuya and Okuku 1994; Bass et al. 2014). Therefore, there is a need to investigate alternative means of control that can reduce

chemical pesticide-related problems (Nahusenay and Abate 2018). Among these, botanical insecticides are an important group of naturally occurring pesticides, which are usually safer for humans, animals, environment and natural enemies than chemical insecticides (Devi et al. 2016).

Currently, the use of plant extracts and essential oils to control insect pests is widely used. Indeed, some plants are chemically protected against insects or contain various biologically active compounds that can be toxic to insect pests, anti-feedants, inhibit growth, inhibit oviposition and even sterilize insects (Sayed et al. 2020). In addition, plant extracts are less toxic to humans, easily degrade in the environment and less persistent than chemical pesticides (Mwine et al. 2011; Silva et al. 2016).

Among the biological active compounds, pyrethrin, which is extracted from *Chrysanthemum* spp. kills insects at low concentrations and has many others advantages such as rapid degradation and is less toxic to beneficial insects (Shawket et al. 2011). Many other plants like neem (*Azadirachta indica*), tinjot (*Otostegia integrifolia*), crinum (*Crinum ornatum*) and common sage (*Salvia officinalis*) have been used to control insects (Nahusenay and Abate 2018; Sayed et al. 2020). Unfortunately, insecticidal effects of aqueous extracts of many others plants, such as pot marigold (*Calendula officinalis*), mint (*Mentha viridis*) and rosemary (*Salvia rosmarinus*) on aphids and the most effective concentrations are unknown.

In this study, the biological parameters of *M. persicae* under laboratory conditions and pesticidal effects of

aqueous extracts of *C. officinalis*, *M. viridis* and *S. rosmarinus* applied as a foliar spray at three different concentrations were investigated.

Materials and Methods

Insects and plants

M. persicae collected from pepper plants cultivated in greenhouses at the National Organic Agriculture Centre, Unaiza, Kingdom Saudi Arabia, in January 2020. This aphid was reared on pepper plants in Plexiglas cages (50 × 50 × 50 cm) at 25 ± 2 °C, relative humidity of 60 ± 10% and a photoperiod of 14 L: 10 D h for several generations (60 days) before use in the experiments. The cultivar of pepper used was Shakira. Pepper plants (Cultivar: Shakira) were reared in the laboratory at day/night temperature ranging from 18 to 25 °C, 60–80% RH, and under ambient light conditions. Plants at eight leaf stage of development, were transferred to 500 mL pots containing a substrate consisting of 1/3 sand and 2/3 peat in a greenhouse at 25 ± 2 °C, relative humidity of 60 ± 10% and a photoperiod of 14 L: 10 D h and were watered on alternate days and no pesticides were used during the experiments.

Infestation rate and population parameters of *M. persicae* on pepper

At the twelve-leaf stage, wingless adult aphids were collected from the colony and transferred to pepper plants in a greenhouse. Fifteen plants were each infested with four adult aphids. The experiment was replicated 3 times and total number of plants used was 45 plants. Data collection started after 2 days after infestation and total number of leaves, infested leaves, and aphid number/cm² of leaf were counted every seven days over a period of two months. The mean relative growth rate (MRGR) and generation time (T) of *M. persicae* were determined according to Leather and Dixon (1984), and the F1 and F2 formulas of Ramade (2003):

$$\text{MRGR} = (\ln N(t_n) - \ln N(t_{n-1})) / (t_n - t_{n-1}) \quad (\text{F1})$$

$$T = \log_2 / \text{MRGR} \quad (\text{F2})$$

where $N(t_n)$ is aphid number/cm² of leaf at time t_n , $N(t_{n-1})$ is aphid number/cm² of leaf at time t_{n-1} .

Plant samples and extracts

Fresh leaves of marigold, Mint and rosemary were collected in March 2020 from plants growing in natural habitats at Al-Qassim as indicated in Table 1. Collected leaves were washed with water and dried, well ventilated in the shade for two weeks (Sarwar 2015). The dried leaves were cut and ground to a fine powder using an electrical grinder. Thirty percent stock solution was prepared for each plant separately. The mixtures were stirred thoroughly with a repeated agitation at 3 h intervals for

24 h. Three concentrations (15, 30, and 45%) were prepared from the final extracts.

Table 1 Description of the three plants tested in the present study.

No.	Common name	Scientific Name	Family name	Part used
1	Marigold	<i>Calendula officinalis</i>	Asteraceae	Leaves
2	Mint	<i>Mentha viridis</i>	Lamiaceae	Leaves
3	Rosemary	<i>Salvia rosmarinus</i>	Lamiaceae	Leaves

Bioassay

To determine the insecticidal effect of aqueous extracts of selected plants, three leaves of each plants were marked and number of *M. persicae* on each marked leaf was counted 1 hour before bioassays. Using a hand-held sprayer, aphids on pepper plants were sprayed with different concentrations (C1 = 15%, C2 = 30%, and C3 = 45%) and the control (untreated) with water. Three infested plants were used for each concentration. Three, six, nine and 12 days after spraying the number of living aphids on each of the marked leaves was counted. Mortality of *M. persicae* population after 12 days were determined and the corrected efficacy percentage was calculated using Henderson and Tilton (1955) formula:

Corrected efficacy (%) = 1 – (n in Co before treatment × n in T after treatment / n in Co after treatment × n in T before treatment) × 100. Where: n = number of *M. persicae*/selected leaf, T = Treated and Co = Control.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS (2015) software program, version 23. Separation of means separation was done using DMRT (Duncan's multiple range tests) test (P < 0.01).

Results

Percentage infestation and population parameters of *M. persicae* on pepper

The percentage infestation and mean relative growth rate of *M. persicae* on pepper plant cultivar Shakira under laboratory conditions are shown in Table 2. During this experiment, total number of leaves/pepper plant was 52.6 ± 13.8 and total number infested 39.8 ± 7.24. Per-

Table 2 Biological parameters of *Myzus persicae* on pepper plants in green house.

Parameters	Average ± SD
Total leaves number	52.6 ± 13.8
Infested leaves number	39.8 ± 7.24
Infestation rate (%)	76.9 ± 9.4%
Aphid number/leaf cm ²	11.24 ± 2.3
Mean relative Growth Rate (MRGR)	0.062 ± 0.007
Generation time (T)	11.12 ± 1.42 days

centage infestation was $76.9 \pm 9.4\%$. The aphid population survey resulted in an average of 11.24 ± 2.3 aphids/cm² of leaf, mean relative growth rate of 0.062 ± 0.007 and generation time of 11.12 ± 1.42 days.

Effect of aqueous extracts on *Myzus persicae* population

Aphid numbers/cm² of leaf on treated and none treated pepper plants were recorded 3, 6, 9 and 12 days after

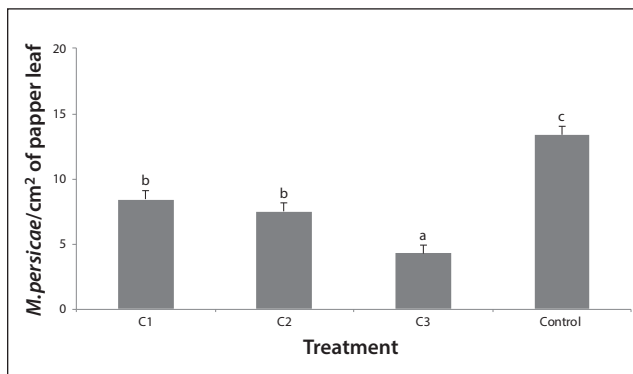


Fig. 1 Effect of an aqueous extract of marigold aqueous on the numbers of *Myzus persicae*/cm² of leaf of pepper plants. (Means followed by the same letter are not significantly different ($\alpha < 0.01$, Duncan's multiple range test).

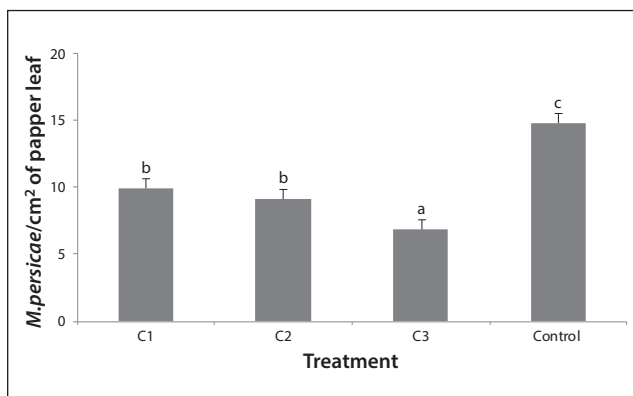


Fig. 2 Effect of an aqueous extract of mint on the numbers of *Myzus persicae*/cm² of leaf of pepper plants. (Means followed by the same letter are not significantly different ($\alpha < 0.01$, Duncan's multiple range test).

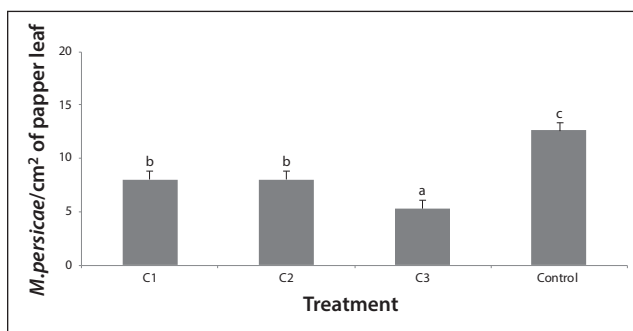


Fig. 3 Effect of an aqueous extract of rosemary on the numbers of *Myzus persicae*/cm² of leaf of pepper plants. (Means followed by the same letter are not significantly different ($\alpha < 0.01$, Duncan's multiple range test).

spraying. Results indicate that all treatments decreased the numbers/cm² compared to untreated plants. Aphid numbers/cm² of leaf after treatment with C1, C2 and C3 of the *C. officinalis* extract were 8.37 ± 1.33 , 7.43 ± 1.46 and 4.33 ± 1.24 , respectively, and 13.37 ± 2.81 for untreated plants. That is, all concentrations in this treatment resulted in significant reductions ($F = 48.57$; $\alpha < 0.01$) in the numbers/cm² (Fig. 1). For *M. viridis* extract, the numbers were 9.95 ± 1.12 , 9.11 ± 1.86 , 6.83 ± 1.66 and 14.81 ± 1.21 , respectively, and this treatment resulted in a significant reduction in the number of aphids ($F = 43$; $\alpha < 0.01$) (Fig. 2). A reduced number/cm² was also recorded after treatment with the extracts of *S. rosmarinus*: 8.05 ± 2.31 , 7.79 ± 0.98 and 5.27 ± 1.32 , respectively, which are significantly different from the 12.61 ± 1.93 recorded on untreated plants (Fig. 3) ($F = 39.76$; $\alpha < 0.01$).

Myzus persicae mortality and efficacy of plant extracts

The efficacy of the different concentrations of aqueous extracts of *C. officinalis*, *M. viridis* and *S. rosmarinus* plants in killing *M. persicae* was determined. Results in Table 3 indicate that the treatments resulted in from 38.24 to 69.82% mortality. The percentage efficacy ranged between 32.41 ± 1.23 and 61.71 ± 4.46 . The highest mortality ($69.82 \pm 5.23\%$) and efficacy ($61.71 \pm 4.46\%$) were recorded for treatment with C3 of the aqueous extract *C. officinalis* (Table 3). Whereas, the lowest mortality ($38.24 \pm 2.42\%$) and efficacy ($32.41 \pm 1.23\%$) were recorded for the treatment with C1 extract of *M. viridis*. Treatments with the three different concentrations of the extracts of *C. officinalis*, *M. viridis* and *S. rosmarinus*, resulted in a significant difference ($\alpha < 0.01$) in mortality and efficacy (Table 3). No significant differences ($\alpha > 0.01$) in mortality and efficacy were recorded for the treatments with C1 and C2 of *C. officinalis*, *M. viridis* and *S. rosmarinus* (Fig. 4, 5), but a significant higher ($\alpha < 0.01$) mortality and efficacy was recorded for the C3 extract of *C. officinalis* treatment compared to that of *M. viridis* and *S. rosmarinus*.

Table 3 *Myzus persicae* mortality and efficacy obtained across treatments with concentrations of extracts of selected plants.

Selected plant	Concentration	Mortality (%)	Efficacy (%)
<i>C. officinalis</i>	C1	$41.76 \pm 2.15c$	$39.53 \pm 2.42c$
	C2	$48.20 \pm 3.45b$	$43.64 \pm 2.57b$
	C3	$69.82 \pm 5.23a$	$61.71 \pm 4.46a$
<i>M. viridis</i>	C1	$38.24 \pm 2.42c$	$32.41 \pm 1.23c$
	C2	$43.34 \pm 3.74b$	$38.74 \pm 0.98b$
	C3	$56.66 \pm 4.37a$	$51.86 \pm 1.74a$
<i>S. rosmarinus</i>	C1	$42.71 \pm 3.35c$	$37.23 \pm 0.79c$
	C2	$46.33 \pm 2.53b$	$40.85 \pm 1.37b$
	C3	$61.76 \pm 4.46a$	$56.81 \pm 1.67a$

Means followed by the same letter are not significantly different ($\alpha < 0.01$, Duncan's multiple range test). C1, C2 and C3: Concentrations.

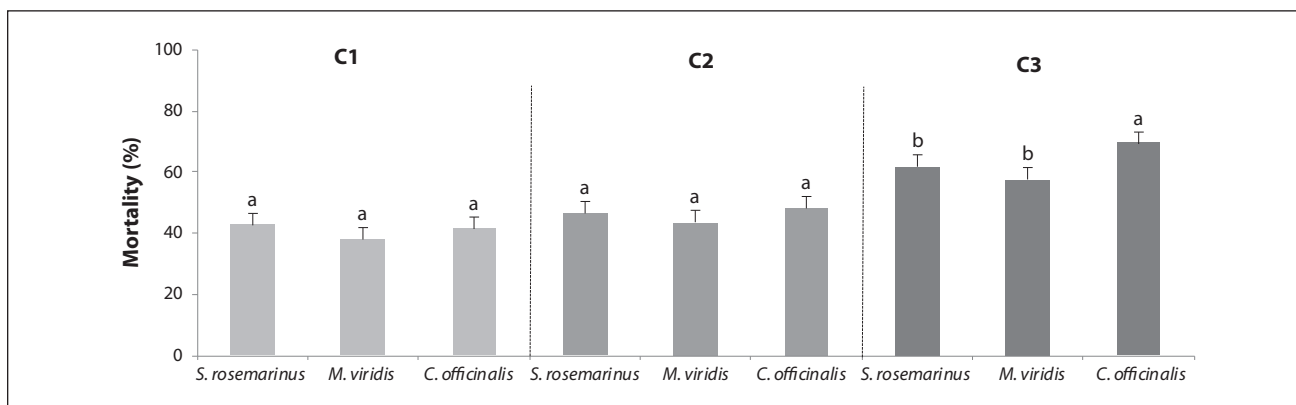


Fig. 4 Percentage mortality of *Myzus persicae* recorded treatments using three different concentrations of aqueous extracts of three species of plants. (Means followed by the same letter are not significantly different ($\alpha < 0.01$, Duncan's multiple range test).

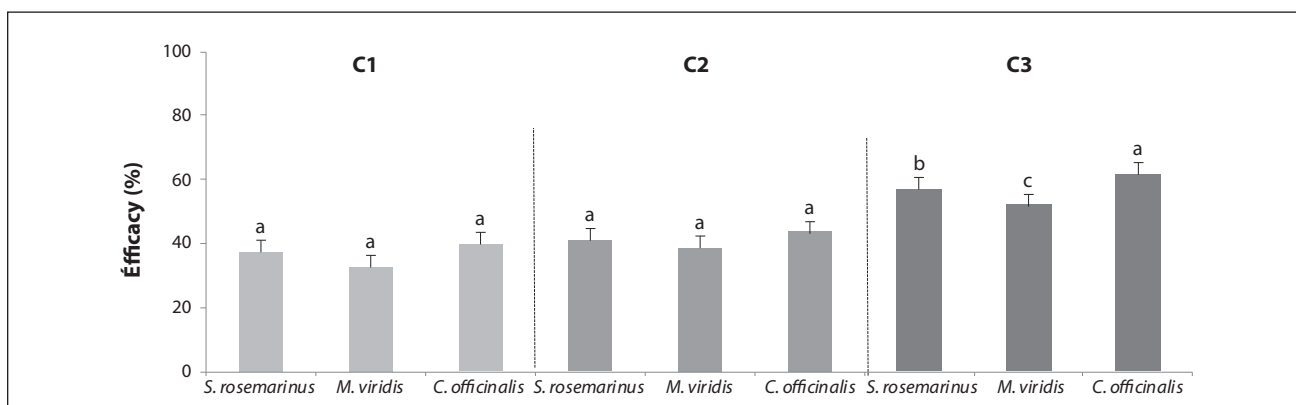


Fig. 5 Efficacy of three concentrations of aqueous extracts of three different species of plants in reducing the abundance *Myzus persicae* on pepper plants. (Means followed by the same letter are not significantly different ($\alpha < 0.01$, Duncan's multiple range test).

Discussion and Conclusions

Many species of plants contain bioactive compounds that are used as botanical insecticides for controlling pests. These botanical insecticides are naturally safe and harmless for consumers, but for insects are toxic, repellent, antifeedants or growth regulators (Sertkaya et al. 2010; Erdogan and Yildirim 2016). Of the insects, *M. persicae*, is classified as a serious pest of pepper plants worldwide due to its high potential growth rate.

In this study, percentage infestation and fitness of *M. persicae* were determined. Results indicate that *M. persicae* have a high reproductive potential on pepper, with a mean relative growth rate and a generation time of 0.062 and 11.12 days, respectively. Results also indicate a high percentage infestation ($76.9 \pm 9.6\%$). Fast development of *M. persicae* on pepper is reported with the generation time ranging between 10.23 and 13.51 days at temperatures between 20 and 25 °C (Satar et al. 2008).

Similarly, Ali et al. (2021) report that *M. persicae* develops faster on pepper (9.96 days) than on cabbage (14.2 days) or crown daisy (10.9 days). In addition, Mdellel et al. (2019) reports that the MRGR of *M. persicae* on pepper at 25 °C ranges between 0.046 and 0.068 depending on soil fertility. Several factors can influence the population growth of *M. persicae*, such the nitrogen level in the soil,

which has a direct effect on host plant quality, which affect the growth rate of *M. persicae* (Mdellel and Ben Halima 2014). Similarly, Dixon (1987) shows that nitrogen is an important factor affecting the fitness of aphids.

The bioassays of the effectiveness of extracts of three species of plants (*C. officinalis*, *M. viridis* and *S. rosmarinus*) in reducing the abundance of *M. persicae* on pepper plants revealed they were effective. Extracts at the highest concentration, C3, in all treatments was the most effective. Of the plant extracts, that of *C. officinalis* was more effective in reducing the abundance of *M. persicae* than that of *M. viridis* and *S. rosmarinus*. This might be attributed to differences in the insecticidal effects on aphids of the chemicals in these plants.

There is a report that flowers of *C. officinalis* contain flavonol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins and a sesquiterpene glucoside (Ukiya et al. 2006). These compounds are highly toxic to plant sucking insects such as aphids and whiteflies and can inhibit feeding and growth of insect pests (Jankowska and Wilk 2011; Murrovhi et al. 2020). The other plant extracts (*M. viridis* and *S. rosmarinus*) were also effective in reducing the abundance of *M. persicae*. Samarasekera et al. (2008) report that the essential oil of *Menthus* spp. contains menthol which can adversely affect insects. Similarly, Ebadollah et al. (2020)

report that essential oils isolated from *Menthus* plants can adversely affect insects and could act as a repellent and antifeedant. In addition, same authors indicate that essential oils of *Menthus* spp. and other Lamiaceae contain a monoterpenoid component, which damages pests.

Studies on *Rosemarinus* reveal that it contains volatile compounds, which can be extracted and used as an effective fumigant against various insects such as coleopteran (*Tribolium* spp.; *Callosobruchus chinensis* (L.)) and lepidopteran pests like *Cadra cautella* (Walker) (Lee et al. 2002; Isikber et al. 2006). Terpenes and monoterpenes of *R. officinalis* affect the central nervous system of insects by inhibiting acetylcholinesterase enzymes (Krzyzowski et al. 2020).

Our study indicates that extracts of three plants can markedly reduce the abundance of *M. persicae* on pepper plants, especially the highest concentration extract of *C. officinalis*, and consequently reduce the damage caused by this pest. Thus, plant extracts could potentially be used to control aphid pests on different crops and as an effective biological control agent for use in an integrated management strategy for controlling aphids.

Further studies on the chemical components of the extracts of the plants tested are needed in order to determine how they affect insects and their effect on the yield and quality of crops. In conclusion, the present study confirms that the potential growth of *M. persicae* on pepper plants is very high and likely to result in serious damage. Extracts of three different species of plants namely: *C. officinalis*, *M. viridis* and *S. rosmarinus*, at three different concentrations, increased the mortality of this aphid. All the plant extracts tested were effective in reducing aphid populations. The extract of *C. officinalis* was more effective than that of *M. viridis* and *S. rosmarinus*. The highest concentration of all extracts, especially that of *C. officinalis*, were effective in reducing aphid numbers and thus in decreasing the damage done by this pest. Therefore, it is recommended that these plant extracts are used to manage aphid abundance instead of chemical insecticides. The use of these plant extracts can reduce the cost and minimize the negative effects of chemical pesticides on consumers and the environment.

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