In vitro and In vivo Evaluation of a Plant Origin Acaricide and In vitro Evaluation of Plant Extracts Against Two-Spotted Spider Mite, Tetranychus urticae Koch

التقييم المخبري والحقلي لمبيد أكاروسي من أصل نباتي والتقييم المخبري للمستخلصات النباتية على الأكاروس العنكبوتي ذو البقعتين، Tetranychus urticae Koch

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Abstract

This study was performed at Sweida Research Center/GCSAR/ in Syria during 2016. The effect of garlic and soft wheat crude extracts as well as the effect of matrine (plant-based acaricide), jolly and sanmite (chemical-based acaricides) were tested under in vitro conditions on two-spotted spider mite, Tetranychus urticae Koch (TSSM). In addition, acaricides used were evaluated under in vivo conditions. Survival % of TSSM was calculated, neglog transformed and subjected to ANOVA analysis using Tukey HSD test. In vitro bioassay results revealed a significant effect of garlic and wheat extracts as well as matrine, jolly and sanmite. After 72 hours, no difference was observed between plant extracts, and among tested acaricides. In vivo bioassay results also showed a significant effect of acaricides used where jolly was the best and matrine and sanmite were comparable. These findings support the idea of using plant-based acaricides (including lectin-based acacicides) as an alternative strategy of using chemical-based acaricides. Taking into account the advantages of in vitro bioassays and based on the results of this study, we suggest predicting in vivo response from in vitro results although this issue needs to be tested first for the crude plant extracts to evaluate their stability under in vivo conditions.

Keywords: Spider Mite, Plant Extract, Matrine, Survival %, Lectin.

ملخص:

نُفذت هذه الدراسة في مركز بحوث السويداء/ الهيئة العامة للبحوث العلمية الزراعية، سورية خلال العام 2016. وجرى اختبار تأثير المستخلص الخام لكل من القمح الطري والثوم، بالإضافة إلى الماترين (مبيد أكاروسي من أصل نباتي) والجولي والسانمايت (مبيدات أكاروسية من أصل كيميائي) على الأكاروس العنكبوتي ذي البقعتين Tetranychus urticae Koch تحت الشروط المخبرية. بالإضافة لذلك، جرى تقييم المبيدات الأكاروسية المختبرة تحت الشروط الحقلية. حُسبت % النجاة للأكاروس العنكبوتي ذي البقعتين، ثم حُولت البيانات وعُرِّضت لتحليل التباين باستخدام اختبار Tukey HSD. بينت نتائج الاختبارات الحيوية المخبرية وجود تأثير معنوي لمستخلصي الثوم والقمح بالإضافة إلى الماترين والجوليوالسانمايت. لم تُلاحظ وجود فروقات فيما بين المستخلصات النباتية المستخدمة، وفيما بين المبيدات الأكاروسية المستخدمة بعد 72 ساعة. كما بينت نتائج الاختبارات الحقلية وجود التأثير المعنوي للمبيدات المستخدمة حيث كان المبيد جولي الأفضل، بينما كان تأثير الماترين والسانمايت متشابها. تدعم هذه النتائج فكرة استخدام المبيدات ذات الأصل النباتي (بما فيها المبيدات ذات الأصل اللاكتيني) كإستراتيجية بديلة عن استخدام المبيدات ذات الأصل الكيميائي. آخذين بالاعتبار إيجابيات الاختبارات المخبرية ونتائج هذه الدراسة، نقترح إمكانية التنبؤ عن الاستجابة في التجارب الحقلية انطلاقا من نتائج التجارب المخبرية على الرغم أن هذا التنبؤ يحتاج لاختبار فيما يخص تأثير المستخلصات النباتية الخام لتقييم ثباتها تحت الشروط الحقلية.

الكلمات المفتاحية: الأكاروس العنكبوتي، المستخلص النباتي، ماترين، % النجاة، اللاكتين.

Introduction

Secondary pest outbreak is caused by using broad-spectrum insecticides that disrupt natural pest control due to the toxicity of these insecticides on non-target biological enemies. This is the case for two-spotted spider mite (TSSM), Tetranychus urticae (Koch). TSSM has a wide range of hosts (more than 1200 species), 150 of them are economically significant (Zhang, 2003).The population of these mites can reach high density quickly, and subsequently reduces the quality and quantity of crops especially after sever infestations.

Mites feed on the leaves by sucking the plant sap. As a result, the photosynthetic efficiency decreases due to the loss of chloroplasts and this eventually will lead to leaf death (Tanigoshi et al. 2004).

As conventional insecticides are used in several agro-ecosystems (Sarwar, 2015) and because TSSM has turned to be a key pest at present, agriculture is facing a serious problem. Spider mites have developed resistance to pesticides rapidly where resistance to over 80 pesticides covering most major chemical groups has been reported (APRD, 2007).It is worth mentioning that TSSM populations (and other mites) have the highest occurrence of pesticide resistance among arthropods in agricultural habitats (Van Leeuwen et al., 2015). In addition, some of insects' biological enemies are sensitive to pesticides (Zanuncio et al., 1998), which will decrease their efficiency for biological control (Biondi et al., 2015). These concerns have directed researchers' attention to search for alternative control methods such as natural pesticides derived from plants (Isman, 2006). Plant extracts are one of the non-chemical control options that have recently received more attention. There are several reports on botanical acaricides proved to be effective against TSSM like neem (Martinez-Villar et al., 2005) and garlic (Boyd and Alverson, 2000).

Biological control and plant-based pesticides are important for developing an Integrated Pest Management Program (Jansen, 2013). Hence, using plant-based pesticides could be an effective strategy to control pests and reduce negative effects of synthetic pesticides.

Therefore, this study focuses on the effect of three acaricides (one of them is derived from plant origin) on TSSM under laboratory and field conditions as well as the effect of two plant extracts under laboratory conditions. In addition, this study addresses the possibility of predicting the in vivo response starting from the in vitro results.

Methods and materials

This study was performed at Sweida Research Center/ GCSAR/ Syria during 2016. The in vivo bioassay was achieved in a homogenized apple field with the following characteristics: soil: loamy clay, apple cultivar: Starkrimson (the age is unified: 30 years) grifted on the rootstock MM 109, altitude: 1550 meter above sea level, average of annual precipitation: 550 millimeter, the weather is hot, dry in summer, and cold in winter.

Preparation of tested acaricides and plant extracts

The toxicity of certain acaricides (matrine is a plant-based acaricide whereas jolly and sanmite are chemical-based acaricides), along with water as a control was tested using the manufacturer recommended concentrations (Table 1) under in vitro and in vivo conditions. In addition, the effect of garlic and soft wheat crude extracts was also tested under in vitro conditions where extraction buffer (0.2 M Nacl) was used as a control. Ten grams from each of the garlic gloves and wheat grains (Bohuth 8: soft wheat) were homogenized in 80 ml of extraction buffer following the ratio 1 to 8 (w/v) (Hou et al., 2010). The homogenates were left at room temperature for 24 hours (H) with stirring several times. Afterwards, the homogenates were filtered using a filter paper, and the resultant solutions were kept at 4° C until use.

Table 1.

Acaricides used and their application rate

Trade name	Effective ingredient	Application rate
Matrine	Matrine 0.5%	100 cm3/ 100 L water
Jolly	Fenbutatin oxide 50%	100 cm3/ 100 L water
Sanmite	Pyridaben 20%	100 g/ 100 L water

Preparation of TSSM

TSSMs were collected from several apple orchards at Sweida Research Center/GCSAR, where no pesticides were used. The mites were brought into a greenhouse and were released on potted kidney bean plants (Phaseolus vulgaris L).

In vitro bioassay

The in vitro bioassay was performed following the method of Sikha et al (2011) with some minor modifications. Complete randomized design with six replications was used for this assay. Apple leaf disks (2 cm diameter) were immersed in each of the different solutions used, and immediately air-dried for ½ H. After that, leaf disks were put in petri plates (9 cm diameter) on wet cotton to keep them turgescent. TSSM adults were collected from the culture and moved to the leaf disks/ 10 mites on each leaf disk/ 3 leaf disks per plate/ 6 plates per treatment at room temperature. Mites regularly observed after 24, 48 and 72H by stereomicroscope. Mites were considered dead when not showing any movement.

In vivo bioassay

Randomized complete block design with three replications was used for the in vivo bioassay. Applied treatments were the three tested acaricides and water as a control. TSSM population (adults and nymphs) was registered on 3 replicates/ 3 trees per replicate/10 leaves per tree (from different parts of the canopy) for each treatment before the spray in addition to 3, 7, 10, 15 and 21 days after spraying. The leaves were passed through a mite-brushing machine and afterwards placed onto a circular glass plate that is coated with a thin layer of glycerol to catch the mites (Henderson and Mc Burnie, 1943).

Data collection and analysis

For both bioassays, the number of living adults (moving stages: adults and nymphs for the in vivo bioassay) was converted into survival % which subsequently neglog transformed (Whittaker et al., 2005) and finally subjected to analysis of variance (ANOVA).Neglog transformation is normally used to reduce the heterogeneity of the data especially when performing bioassays and using percentage data. This method has been used before (Belay et al., 2018). Survival %was expressed as means ± standard deviations. The means were compared using Tukey HSD test at 0.01 probability level for the in vitro bioassay and at 0.05 probability level for thein vivo bioassay by using the SPSS program version 19.

Neglog = (sign X) * Ln (|X| + 1). X is survival %.

Results and discussion

Effect of acaricide and plant extract treatments under in vitro conditions

The effect of several acaricides including matrine (a natural-based acaricide extracted from wild medicinal plant, Sophora flavescens Ait), jolly and sanmite (chemical-based acaricides) was evaluated under in vitro conditions at different time points. ANOVA results showed a significant effect of acaricide application on the survival % of TSSM as compared to the control (Fig 1, Table 2). No difference was observed after 24 H among tested acaricides whereas only matrine reduced the survival % significantly compared to the control after 48 H. All tested acaricides showed significant differences compared to the control after 72 H but no difference was registered among them (Fig 1).

The survival % of the control treatment (only water) was registered 91%, 88%, 78% after 24H, 48H and 72H, respectively (Fig 1).

Table 2.

Mean square of survival %(transformed data) for tested acaricides under in vitro conditions after 24H, 48H and 72H

Source of variance	d.F	24H	48H	72H
Treatment	3	0.028	0.786**	1.176**
Error	20	0.01	0.01	0.073

**: indicates significant differences at P-value < 0.01

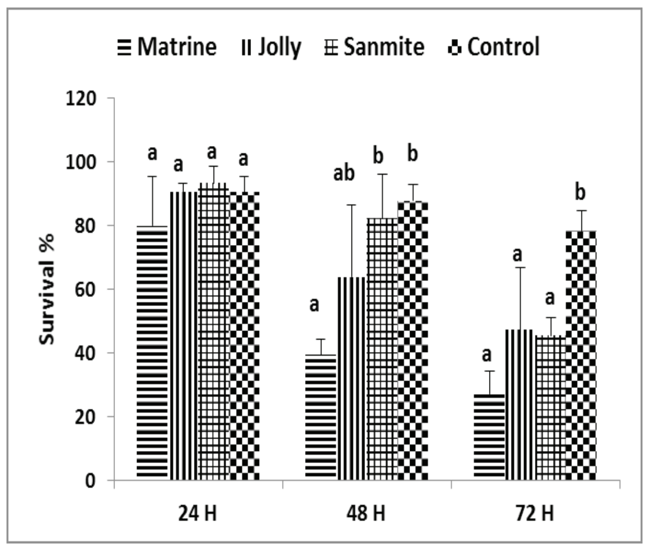

Fig. 1.

Survival % of TSSM adults after immersing leaf disks in tested acaricides: matrine, jolly, sanmite and water as a control. The survival % after 24hours (H), 48 H and 72 H is shown. Bars represent means ± standard deviation based on 6 replicates (plates)/three leaf disks per plate/10 adults per leaf disk. Within each group (24H, 48H or 72H) different letters indicate significant differences at P-value < 0.01 (Tukey test).

Crude extract effect for garlic gloves and soft wheat grains was also tested under in vitro conditions. Both extracts had significant effect compared to the control especially after 72H although no difference was observed between the two tested extracts (Fig 2, Table 3). After 24H, the effect of tested extract was comparable to the control, but only garlic extract shows significant effect on TSSM survival % after 48H proving its rapid efficacy compared to wheat extract (Fig 2).

The survival % of the control treatment (0.2 M Nacl) was registered 94%, 78%, 56% after 24H, 48H and 72H, respectively (Fig 2). This reduction can be explained by the effect of Nacl, which is apparently greater than the effect of only water especially after 72H (56% for the salt treatment and 78% for water treatment). The reduction of survival % in both controls might also be due to the insufficient food (only 2 cm diameter leaf disks were used). In addition, the in vitro bioassay was performed at room temperature, which is not optimum for TSSM. Under field conditions, the average maximum temperature in June, July and the first half of August (2016) was 30°±2.4° C (Climate Measuring Station/ Sweida Research Center).

The effectiveness of garlic and wheat crude extracts is most probably due to the effect of lectins, even though the effect of other components cannot be excluded. The activity of lectins, which are carbohydrate-binding proteins, against insects was proven and received a lot ofattention (Vandenborre et al., 2011; Bharathi, 2017).Constitutively expressed lectins are often concentrated in seeds or vegetative storage tissues, and they probably act as storage proteins (Van Damme et al., 1998). However, in case of pest attack they play a role as defense-related proteins against pathogens and insects (Roy et al., 2014). Garlic gloves and wheat grains contain lectins called Allium sativum agglutinin, and wheat germ agglutinin, respectively. Their insecticidal activity has been reported in transgenic plants (Sadeghi et al., 2007), and by using the lectin in artificial diets (Harper et al., 1998), or by using the purified lectin in bioassays (Roy et al., 2008).

Table 3.

Mean square of survival % (transformed data) for tested plant extracts under in vitro conditions after 24H, 48H and 72H

Source of variance	D.F	24H	48H	72H
Treatment	2	0.014	0.191**	0.971**
Error	15	0.005	0.026	0.077

**: indicates significant differences at P-value < 0.01

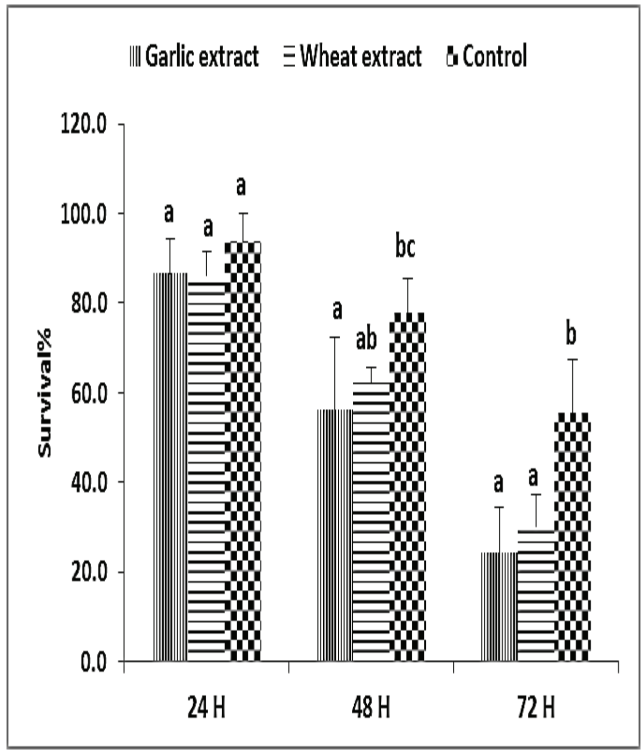

Fig. 2.

Survival % of TSSM after immersing leaf disks in tested plant crude extracts: garlic extract, wheat extract and 0.2M Nacl as a control. The survival % after 24H, 48H and 72H is shown. Bars represent means ± standard deviation based on six replicates(plates)/ three leaf disks per plate/ 10 adults per leaf disk. Within each group (24H, 48H or 72H) different letters indicate significant differences at P-value < 0.01 (Tukey test).

Effect of acaricide treatments under in vivo conditions

The acaricide treatments were effective in controlling TSSM population under in vitro conditions. Therefore, a bioassay was conducted to evaluate their effect under field conditions, and to compare the effect of the plant origin acaricide with the chemical-based acaricides. The results revealed a significant reduction in survival % of TSSM moving stages at 3 days (D), 7D and 10D after spraying for all tested acaricides(Fig 3, Table 4). At the three mentioned time points, Jolly was the most effective acaricide against moving stages of TSSM (survival %: 5.1%, 2.6%, 0.4% at 3D, 7D, 10D respectively, P-value < 0.05). It is not surprising that jolly was more effective than matrine under field conditions (they were comparable after 72H under in vitro conditions, Fig 1) because only adults were observed for thein vitro bioassay whereas moving stages (adults and nymphs) were registered for thein vivo bioassay. Additionally, both bioassays were assessed at different durations (3 days for the in vitro bioassay and 21 days for the in vivo bioassay).

In general, using matrine is safe to beneficial arthropods and to the environment (Wang et al., 2012) because its plant origin. In addition, registering no significant difference between matrine and sanmite (Fig 3) supports the idea of using matrine instead of synthetic pesticides. Furthermore, the effective ingredient of matrine is very low 0.5% compared to 50% of jolly and 20% of sanmite (Table 1). This means that there is a possibility to increase the effective ingredient of matrine to be more efficient although this issue has to be proven first. Matrine is a quino lizidine alkaloid extracted from Sophora flavescens (Liu et al., 2008). In accordance with our results, matrine has been reported to be effective against mites (Niu et al., 2014).Taking into account the high toxicity of chemical origin pesticides towards the environment, biological enemies, animals (Mahmood et al., 2016) and human beings (Nicolopoulou-Stamati et al., 2016), there is a continual need to use safer alternatives particularly plant origin pesticides.

Table 4.

Mean square of survival % (transformed data) for tested acaricides under in vivo conditions 3, 7 and 10 days after spraying

Source of variance	D.F	3D	7D	10D
Treatment	3	4.476*	3.628*	3.760*
Block	2	0.018	0.068	0.017
Error	6	0.064	0.022	0.060

*: indicates significant differences at P-value < 0.05

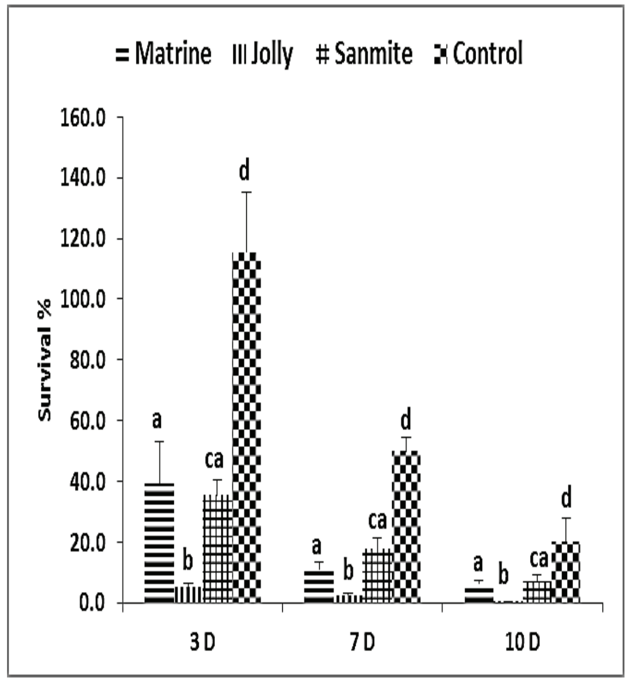

Fig. 3.

Survival % of TSSM for in vivo bioassay after spraying with tested acaricides: matrine, jolly, sanmite and water as a control. The survival % after 3 days (D), 7D and 10D is shown. The average of moving stages number before spraying were 476, 513, 967 and 383 for matrine, jolly, sanmite and control treatments, respectively. Bars represent means ± standard deviation based on 3 replicates/ 3 trees per replicate/ 10 leaves per tree. Within each group (3D, 7D or 10D) different letters indicate significant differences at P-value < 0.05 (Tukey test).

Even though observation was registered, 15D and 21D after spraying, obtained data were not subjected to ANOVA because the mean number of TSSM in the control treatment was reduced naturally (Fig 4). This reduction in TSSM number is probably due to the high maximum temperature registered during the second half of the in vivo bioassay (36.2° ± 0.9° C), whereas it was 30° ± 1.9° C during the first half of the bioassay (Climate Measuring Station/ Sweida Research Center). The field experiment was deliberately started when the number of TSSM reached the highest values at the late season. This may also explain the reduction of TSSM number (control treatment) because TSSMs started entering the diapause period at the late season.

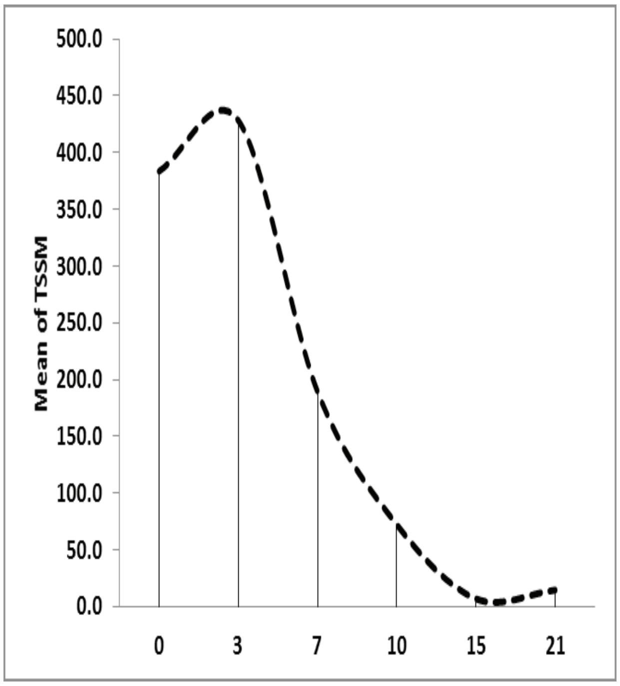

Fig. 4.

Mean of TSSM number (moving stages) in the control based on 3 replicates/ 3 trees per replicate/ 10 leaves per tree for the in vivo bioassay. Horizontal axis represents the time points (in days) of observations.

Prediction of in vivo response from in vitro bioassay results

In vitro bioassays have the potential to yield very important data but extrapolation to in vivo responses remains a major challenge. In vitro bioassays are easy to perform, rather cheap and not time and labor consuming. In addition, large numbers of treatments can be performed in a small place. On the contrary, in vivo bioassays are expensive, difficult to conduct, and time and labor consuming. This encourages researchers to test their treatments under in vitro conditions first especially that in vitro bioassays can screen their treatments before testing them in the field. It has been reported that the results of in vitroare still true for in vivo (Pokle and Shukla, 2015; Reddy et al., 2014).

Back to the issue raised by this study: can in vitro results be generalized to field conditions? Based on the results of this study and on some reports (Mamun et al., 2014), the answer is yes although this issue is still a prediction. At least, treatments can be screened and selected under in vitro conditions before being tested under in vivo conditions.

In the present study, plant extracts proved to be efficient under in vitro conditions but before this result can be generalized to field conditions their stability under field conditions has to be proven. This stimulates further researches to test this issue especially that plant extracts (rich in lectins like garlic and wheat) will pave the way of using acaricides derived from these extracts.

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