J. Plant Prot. and Path., Mansoura Univ., Vol.7 (10), 623– 628, 2016

Laboratory and Semi Field Evaluation of Garlic Aqueous Extract as Acaricide Against Two Tetranychid Mites (Acari: Tetranychidae)

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ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval), are two of the most important mite pests of different host plants in Egypt. These mites are spreading rapidly because of their resistance to acaricides; therefore, it was necessary to develop a new biological control tactics for mite management. Plant extracts, such as garlic extract (*Allium sativum* Linn.), may represent easy, available and viable alternative, because they are considered to be minimum-risk pesticides. A series of laboratory and semi-field experiments were considered to determine the susceptibility of adult mite females to different concentrations of garlic aqueous extract (G.A.E.). Mortality was measured upon treatment with five concentrations ranging from 1 to 25% W/V. Female mortality increased with concentration, *T. urtica*e was more tolerant than *T. cinnabarinus* through laboratory studies as maximum mortality values were 90% and 100% after 24 hours using concentration 25% W/V, respectively. The chemical composition of the *Allium sativum* aqueous extract was characterized by GC/MS analysis which revealed the occurrence of 33 compounds, of which the major compound was identified as 2- Furancarboxaldehyde,5-(hydroxymethyl). The efficacy of G.A.E. was evaluated against *T. urtica*e and *T. cinnabarinus* under semi-field conditions. Highest reduction (89.75 %) in *T. urticae* population was recorded by using concentration 50% W/V one day after treatment (DAT). *T. cinnabarinus* was more tolerant under the semi-field conditions as the reduction was (80.14 %) using the same concentration. G.A.E. is effective in the control of both tetranychids. It is promising candidates for biological control of these two mite pests.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urtica*e Koch and carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) have become important agricultural pests during the last century. In fact, their riskiness is due to having large diversity of host plants; (Migeon and Dorkeld 2007) reported 3877 host species for *T. urtica*e around the world. These pests are usually controlled by synthetic acaricides, which are currently the most effective means of pest control. Indiscriminate uses of these chemicals not only cause adverse effects on mammals' health, but also affected many other non-target organisms. They are also responsible for the development of insecticide-resistance phenomenon. Recently, mite management is difficult, because some populations have developed resistance to the chemical acaricide (Beers *et al*.1998).

Integrated pest management for tetranychid mites largely depended on the use of a few selective acaricides (Van Pottelberge *et al.* 2009). Thus, the new approach is to reject or minimize the use of chemical insecticides both for their inefficiency and for preventing ecological system damage. So, it is important to develop new methods to prevent or at least limit tetranychid mites' attacks, of which natural products may be more useful because of their low level of environmental pollution and low toxicity to human (Liu *et al.* 2000).

Garlic (*Allium sativum* L.), was used by Dabrowski and Seredynska (2007) as an alternative to synthetic chemical pesticides. They showed the acaricidal properties of garlic aqueous extract to control *T. urtica*e. Also, garlic has been shown to be a repellent for human ticks (Catar 1954) and *T. urticae* mites (Boyd and Alverson 2000; Carlos *et al.* 2008). All these investigations suggest that garlic might be useful for controlling *T. urticae* and *T. cinnabarinus* populations in the field (Singh *et al.* 2001). So far the acaricidial activity of garlic extracts has only received minor attention (Seufi *et al*. 2007). Therefore, the purpose of this study was to evaluate the effectiveness of garlic extracts to control *T. urticae* and *T. cinnabarinus*.

METERIALS AND METHODS

Experimental mite cultures

T. urticae and *T. cinnabarinus* were collected from unsprayed castor bean plants in Egypt. The mites were identified at the Acarology Laboratory, Plant Protection Research Institute, ARC by following the detailed descriptions mentioned by (Zhang & Jacobson 2000; Zhang, 2003). Both mites were maintained on green beans (*Phaseolus vulgaris* L.) leaves upside down on moisten cotton pads in Petri-dishes (12 cm in diameter). All Petri-dishes were placed in a closed box and kept under controlled conditions at 25±2°C, and 16:8 h (L:D) in the Acarology Laboratory. The humidity in the box was kept at 75±5% RH using saturated solution of NaCl. The cotton pads were moistened daily and all the ends of the leaves were covered with wet cotton to avoid leaves dryness and to prevent mite escape. Mites were transferred on fresh cotton leaves every 3 days.

Preparation of aqueous garlic extract

For preparation of garlic aqueous extract (G.A.E.) stock solution (50% W/V). The protective layer of garlic cloves were peeled out, 50 gm of garlic were weighed and rinsed. Using an electric grinder, garlic was crushed and completely blended for two minutes at high speed with 100 ml of distilled water. After 30 minutes the homogenate was then filtered by passage through Whatman's filter paper No. 1. Stock solution was stored in a sterile brown bottle and kept in

refrigerator at 4°C until used (Brooklyn Botanic Garden, 2000 & Iwalokun *et al.*, 2004). Four concentrations of (G.A.E.) 1, 5, 12.5 and 25% W/V were prepared by diluting the stock solution with distilled water.

Garlic (*A. sativum*) used for this study was free of any pre-harvest chemical treatments (organic product). Garlic plants were freshly harvested, stored for three months at room temperature in a dry dark room that has ample air circulation.

Laboratory bioassay procedure

Fresh uninfected green beans leaves were transferred to the laboratory and cleaned by sterilized water. Leaf discs of two cm in diameter were placed upside down on moisten cotton pads in Petri-dishes (9 cm in diameter). Ten adult newly emerged females' aged 0-48 hrs. were placed on each disc. Mites were treated by spraying the prepared aqueous garlic extract concentrations. Control variants were sprayed with sterilized distilled water. The experiments were proved in 5 replicates for each concentration. Bioassays were repeated twice on different days. The bean leaf discs were maintained at room temperature (25±2°C, 60±5% RH). Mite mortality was checked daily for seven days.

Chemical analyses

GC/MS analyses were conducted in Central Agricultural Pesticide Laboratory, ARC using an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 ms (30 m x 0.32 mm x 0.25 µm film thicknesses). Sample was injected under the following conditions. Helium was used as carrier gas at approximately 1.0 ml/min., pulsed split less mode. The solvent delay was 3 min. and the injection size was 1.0 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230 ºC. The electron multiplier voltage (EM voltage) was maintained 1250 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60 ºC (3min) then elevated to 300 ºC at rate if 8 ºC/min. the injector temperature was set at 280 ºC. Wiley and Wiley Nist mass spectral data base was used in the identification of the separated peaks.

Semi field experiments

Green beans (*Phaseolus vulgaris* L.) plants were used as the host plant in the laboratory. Green beans were prepared for planting in pots in open space and then transferred to the laboratory. The plants were separated from each other to prevent touching and movement of mites. Plants were infested by the experimental mites (20 female/ plant). After mite's number reached to 3:4 in one inch, the toxicity of three different concentrations of garlic aqueous extract was examined, each treatment was replicated four times, and distilled water was used as a control. Ten leaves were taken from each replicate and number of mites (immature and adult) was counted before treatment and 1, 3, 7 days post-treatment by the aid of a stereomicroscope.

Statistical analysis

Lethal effect of garlic aqueous extract was evaluated as percentages of cumulative daily mortality, corrected for mortality in the control variant according to Abbott's formula (Abbott, 1925). Lethal effect of the extract was estimated based on median lethal concentration (LC_{50}) after 24 h of treatment calculated by probit analysis according to Finney (1971). LC_{50} was calculated using a computerized software program (LdP line) (a copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt). Confidence intervals of varying LC_{50} values were calculated at P = 0.05.

Toxicity index (Ti) was calculated according to Sun (1950) equation using LdP line program as follow:

$$\mathbf{Ti = \frac{LC_{50}\ of\ the\ most\ toxic\ effect\ of\ (G.A.E.)}{LC_{50}\ of\ less\ toxic\ effect\ of\ (G.A.E.)}\ X\ 100}$$

The efficacy of pesticides (Ef %) was calculated by Henderson-Tilton's formula

$$\mathbf{Ef\% = [1 - (\frac{n\ in\ co\ before\ treatment\ x\ n\ in\ T\ after\ treatment}{n\ in\ co\ after\ treatment\ x\ n\ in\ T\ before\ treatment})] \times 100}$$

Where

n = the number of living mites
t = treated
c = control

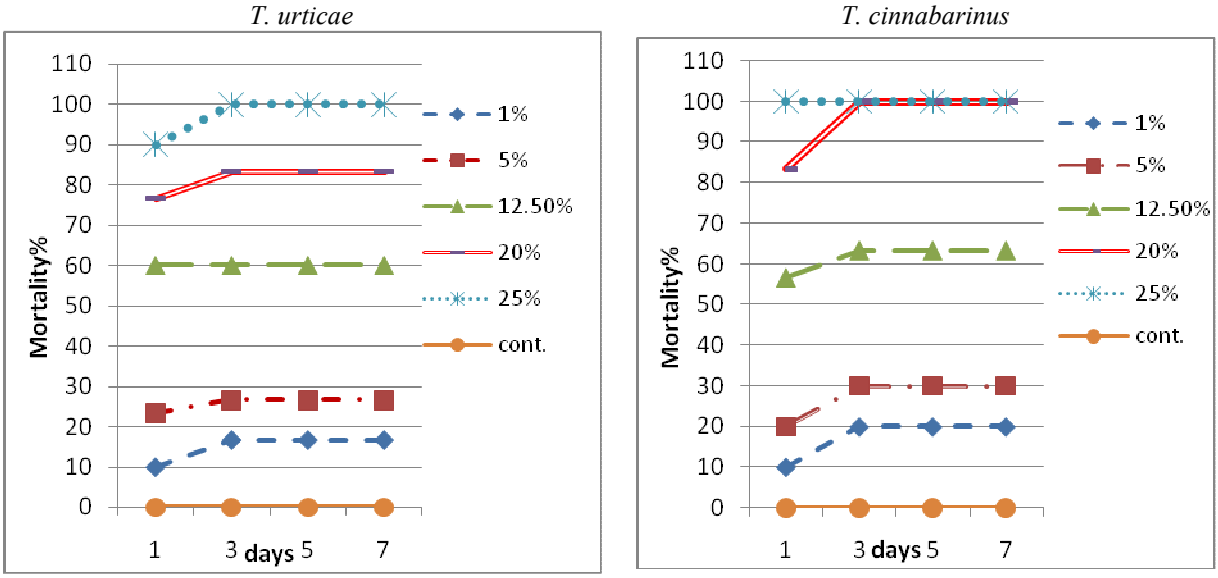
Experimental design

Experimental treatments were arranged in split-split plot design with three replications in laboratory studies and four replications in semi-field studies, where pest treatments (*T. urticae* and *T. cinnabarinus*) were assigned to main plots, while concentrations (Control, 1.0, 5.0, 12.5, 20.0 and 25.0%) in the sub plots and durations (after 1, 3, 5 and 7 days) in the sub-sub plots analysis of variance (ANOVA) according to Snedecor and Cochran (1981); meanwhile untransformed data are presented in this paper.

The mean values of the individual trait were statistically compared using Analysis Of Variance (ANOVA) for each experiment according to Snedecor and Cochran (1981). Before analysis, data were transformed by ($\sqrt{;x}$ + 1.0); meanwhile untransformed data are presented in this paper. Mean comparisons for the studied traits were done according to Duncan's Multiple Range Test at $P \leq 0.05$ (Duncan 1955).

RESULTS AND DISCUSSION

Laboratory evaluation of garlic aqueous extract against *T. urtica*e and *T. cinnabarinus* showed that mortality was high at 24 hrs. and three days after treatment . No effect on mortality percentage was observed at five and seven days post-treatment. No mortality was noticed in control. The acaricidal activity of tested concentrations of (G.A.E.) against adult females of *T. urticae* and *T. cinnabarinus* was enhanced with increasing concentrations, Fig. (1).



*cont. =control

Fig. 1. Cumulative mortality of *Tetranychus urticae* and *Tetranychus cinnabarinus* adult females at different concentrations of garlic aqueous extract over time.

Mortality values caused by (G.A.E.) for *T. urticae* individuals after 24 hours were 10, 23.33, 60, 76.66 and 90% and increased to 16.66, 26.66, 60, 83.33 and 100% after three days for concentrations 1, 5, 12.5, 20 and 25% W/V, respectively (Fig 1).

The same trends were observed when we used concentrations of (G.A.E.) mentioned before against the carmine spider mite. Results illustrated in Fig (1) proved that *T. cinnabarinus* were less tolerant to the extract than *T. urticae* as mortality values were 10, 20, 56.66, 83.33 and 100% after 24 hours increased to 20, 30.66, 63.33, 100 and 100% after three days of treatment with the before mentioned concentrations, respectively.

Toxicity of (G.A.E.) was estimated based on values of the median lethal concentration (LC_{50}) which calculated for mites treated with (G.A.E.) concentrations at p-level < 0.05. Results indicated that *T. urticae* were highly tolerant to the extract than *T. cinnabarinus*. The calculated LC_{50} after 24 hours of treatment were 7.5343% W/V with a toxicity index (Ti= 100) and 8.008% W/V with a toxicity index (Ti= 94.08) for *T. cinnabarinus* and *T. urticae*, respectively (Table 1).

Table 1. Toxicity of garlic extract against adult females of *Tetranychus urticae* and *Tetranychus cinnabarinus*, 24 hours after treatment.

Treatment	Lc_{50} % W/V	Lc_{90} % W/V	Slope	Toxicity index %
Tetranychus cinnabarinus	7.5343	30.139	2.1286±0.3182	100.00
Tetranychus urticae	8.008	40.2011	1.829±0.2872	94.08

The present results are in agreement with Dabrowski and Seredynska (2007) who studied the water extract of *Allium sativum* which showed a strong activity as a feeding suppressant for *T. urticae*, and they recorded that garlic extracts caused 48–57% mite mortality. Attia *et al.* (2011) examined the effect of garlic distillate concentration on its efficacy as a natural pesticide against *T. urticae*, by quantifying the effects of a wide range of distillate concentrations. They mentioned that garlic distillate treatment caused significant *T. urticae* mortality at low concentrations, with LD50 and LD90 values of 7.49 and 13.5 mg/l, respectively. Erdogan *et al.* (2012) investigated the effect of ethanolic extract of *A. sativum* on *T. urticae* where the mortality reached to 78% using concentration 12%. Geng *et al.* (2014) studied the contact toxicity and repellent effects of garlic-straw extracts against adult female of *T. urticae*. They recorded 76.5% mortality of mites using concentration 20 g/L, 48 h after treatment with LC50 value of 7.2 g/L. Repellency was 95.6% after 24 h for the extract concentration 20 g/L. There were no references of other studies using garlic extracts against *T. cinnabarinus*. Indeed, there is a strong possibility that the garlic essential oils have more than one site of action, especially because they contain complex mixtures (Miresmailli and Isman 2006). In future experiments, the influence of the different chemical constituents identified in garlic distillates should be tested on the phytophagous mites.

Thirty three compounds were identified in (G.A.E.) by GC/MS. The main nine components identified are listed in Table (2) according to their retention times and their percentage composition. These compounds comprise 81.5% of the total composition. 2-Furancarboxaldehyde,5-(hydroxymethyl)- was the most abundant compound (58.64%), followed by 3-Vinyl-1,2-dithiacyclohex -5-ene (6.74%), 3-Vinyl-1,2-dithiacyclohex -4-ene(4.68%), Allyltrisulfide (2.5%), Allyl disulfide (2.25%) and Methyl allyl disulfide (2.05%). Similar results were obtained by Mohammed (2013) who recognized 2-Furancarboxaldehyde,5-

(hydroxymethyl)- as the bioactive compound of ethanolic, methanolic and acetone garlic extracts, with concentrations 53.84, 65.67 and 54.30, respectively. Allicin (diallylthiosulfinate) which found in garlic and responsible on its antimicrobial activity is very thermo-labile and produces various disulfide compounds on heating (Han, 1995). Itakura (2001) confirmed that allicin in fresh garlic extracts was decomposed in the injection port of GC to produce vinyldithiins which were detected as major peaks of garlic extracts. Roy *et al.* (2006) also identified diallyl disulfide and vinyldithiin from garlic. Also, the toxic effects of *A. sativum*may be due to the presence of organosulphur components (Singh *et al.* 2001), because some organosulphur compounds have been shown by Prischamann *et al.* (2005) to reduce the population densities of mite pests. Our results indicated the presence of some organosulphur compounds such as Allyl trisulfide, Allyl disulfide, Methyl allyl disulfide and Methyl allyl trisulfide, these results are in agreement with (Roy *et al.* 2006; Attia *et al.* 2011; Mohammed 2013; Wang *et al.* 2016).

Table 2. Main component of garlic aqueous extract identified by GC/MS

No.	Retention time (min)	components	Compound percentage %
1	3.92	2-Furancarboxaldehyde (CAS) Furfural	1.40
2	4.95	Disulfide, methyl 2-propenyl (CAS) Methyl allyl disulfide	2.05
3	8.25	Diallyldisulphide (Allyl disulfide)	2.25
4	8.55	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	1.62
5	9.45	Trisulfide, methyl 2-propenyl (CAS) Methyl allyltrisulfide	1.62
6	10.42	3-Vinyl-1,2-dithiacyclohex-4-ene	4.68
7	10.90	3-Vinyl-1,2-dithiacyclohex-5-ene	6.74
8	11.99	2-Furancarboxaldehyde,5-(hydroxymethyl)-	58.64
9	12.45	Trisulfide, di-2-propenyl (Allyltrisulfide)	2.50

Semi field experiments

The data on the effect of three concentrations of (G.A.E.) against mites, *T. urticae* and *T. cinnabarinus* showed reduction in mite infestation under semi field conditions (Table 3). G.A.E. was found to be potent against both of the two-spotted spider mite, *T. urticae* and the carmine spider mite, *T. cinnabarinus* and effect on mites in a dose dependent manner. As it caused 77.42, 80.58 and 89.75 percent reduction for *T. urticae* at concentrations 12.5, 25 and 50% W/V after 24 hours of treatment, respectively and decreased to 57.66%, 69.08% and 86.16% for concentrations mentioned before, after seven days treatment, respectively. However the reduction percentage decreased when the same concentrations used against *T. cinnabarinus*. Results showed 75.30%, 78.70% and 80.14 % reduction in *T. cinnabarinus* population 24 hours after treatment and decreased to 72.72%, 76.04% and 76.65% seven days after treatment for the same concentrations, respectively when the post treatment count were correlated with pre-treatment (Table 3). These results generally indicated that *T. cinnabarinus* was more tolerant than *T. urticae* in semi-filed conditions. Data obtained revealed that (G.A.E.) appear highly initial kill against both of tested mites. The Green beans plants sprayed with (G.A.E.) recovered and showed better growth than the mite infested plants (control).

There were no references found of other studies using garlic extracts on tetranychid mites in semi-field conditions. However, Hata, *et al.* (2016) assessed the effect of garlic (*Allium sativum* L.) and other aromatic plants on number of two-spotted spider mite (TSSM), *T. urticae*, when intercropped with strawberry in the field. They found that intercropping with garlic caused a greater reduction of *T. urticae* (up to 52 %) in strawberry plants when higher populations of mites occurred in the field. They used three densities of garlic plants (one, two and three rows among the strawberry rows) which gave reduction to the mobile forms of *T. urticae* 49, 53 and 60 % (greenhouse) and 44, 51 and 65 % (field), and eggs by 38, 43 and 64 % (field), respectively. They suggested that intercropping garlic plants between strawberry rows is a promising strategy to reduce TSSM populations.

Table 3. The number of *T. urticae* and *T. cinnabarinus* on Green beans and efficacy of (G.A.E.)**(Ef %) according to Henderson-Tilton formula**

Garlic extract concentration	Mean number (*) of *T. urticae* BT	1DAT	3DAT	7DAT	Ef % 1DAT	3DAT	7DAT
control	3.6 bc	5.9 bc	6.8 bc	22.3 a	-	-	-
12.5% W/V	7.5bc	2.4 c	4.7bc	19.7 a	77.42	61.81	57.66
25% W/V	8.3bc	2.6 c	2.8bc	10.9 b	80.58	77.94	69.08
50% W/V	6.51bc	1.1 c	1.4 c	4.9bc	89.75	88.09	86.16
	Mean number (*) of *T. cinnabarinus*					Ef %	
control	11.5 def	18.9 c	34.4 b	65.2 a	-	-	-
12.5% W/V	8.4 efg	3.1 g	6.3 efg	12.5 cde	75.30	73.46	72.72
25% W/V	12.8cde	4.5 fg	7.9efg	17.1cd	78.70	76.91	76.04
50% W/V	10.1defg	3.4 g	6.7efg	13.4cde	80.14	77.57	76.65

(*) motile forms; BT = before treatment; DAT = days after treatment.
Means followed by the same letter(s) are not significantly differed by the least significant Difference (Duncan, 1955).

Results of analysis of variance in Table (4) showed that there were no significant differences among two pests in laboratory studies, but it showed high significant differences in Semi-field experiments. In respect to different concentration treatments, highly significant differences were detected for the two

experiments, which demonstrated an existence of high effect of different concentrations. With regard to duration there were highly significant differences detected in the two experiments.

In terms of the interaction between pests and concentrations, there were significant differences in laboratory studies, but it was highly significant in Semi-field studies. Concerning the interaction between pests and duration it showed non-significant differences in laboratory studies, but it was highly significant in Semi-field studies. While the interaction between concentration and duration, showed highly significant differences in the two traits. The interaction between the three studied factors (pests, concentration and duration), was significant and highly significant in laboratory and Semi-field studies, respectively.

Table 4. ANOVA for different traits under different pest, concentrations and durations.

SOV	Laboratory studies		Semi-field studies	
	df	Ms	df	Ms
Fac A (pest)	1	5.063^{ns}	1	1941.346^{**}
Error	2	0.646	3	3.164
Fac B (conc.)	5	391.979^{**}	3	1524.322^{**}
AB	5	1.613^{*}	3	861.212^{**}
Error	20	2.929	18	17.006
Fac C (duration)	3	3.063^{**}	3	1475.468^{**}
AC	3	0.174^{ns}	3	136.751^{**}
BC	15	0.246^{**}	9	373.362^{**}
ABC	15	0.190^{*}	9	138.391^{**}
Error	72	0.097	72	8.814

***showed significantly different (P<0.05)**
****showed highly significantly different (P<0.01)**
ns not significantly different

Although few plant-derived essential products are effective in controlling arthropods, but some of those are prohibited because of their potential phytotoxicity. Cloyd *et al.* (2009) studied the phytotoxicity of a solution of garlic essential oil and demonstrated that it is not phytotoxic. Also, garlic extracts were mentioned by Panella *et al.* (2005) and Isman (2008) to be safe and pose few risks to environment, with minimal impact on animal and human health. Nour El-Deen *et al.* (2013) considered garlic-seed extract the most suitable materials for IPM because it caused high mortality for *T. urticae* with minimal effect on its predatory mite *Phytoseiulus persimilis*.

Finally, many essential oils have short residual activity because of temperature and UV light degradation (Miresmailli and Isman 2006). Therefore, aqueous extract of garlic can be useful to control the two-spotted spider mites, *T. urtica*e and carmine spider mite, *T. cinnabarinus* populations on different host plants grown through Integrated Pest Management (IPM) and organic systems of agriculture. But it is important to investigate the possible toxic effects from spraying diluted garlic extract (with repeated applications) in mammals before application on a large scale in integrated pest and disease management programs.

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تقييم فعالية المستخلص المائى للثوم معمليا و نصف حقليا ضد نوعين من الحلم العنكبوتى

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يعد كلا من الحلم القرمزى العنكبوتى والعنكبوت ذو البقعتين من أهم أنواع الأكاروسات فى مصر وحيث أنهما سريعا الانتشار وبسبب مقاومتهما للمبيدات الأكاروسية كان من الضرورى ايجاد حل لمكافحة هذه الآفات مكافحة بيولوجية .وتعد المستخلصات النباتية التى من بينها مستخلص الثوم بديل سهل ومتاح للحد من من أخطار المبيدات. فى هذه الدراسة تم عمل تجارب معملية ونصف حقلية لتحديد مدى تأثر الأنثى البالغة بالتركيزات المختلفة لمستخلص الثوم المائى حيث تم اختبار ٥ تركيزات ، وقد أظهرت النتائج تأثر الأنثى البالغة طرديا بزيادة التركيزات وكذلك حساسية الحلم القرمزى وتأثره عن النوع الآخر. كما تم تحليل المكون الرئيسى لمستخلص الثوم بواسطه الكروماتوجرافى و اللذى أثبت وجود ٣٣ مركب. و دراسة تأثير المستخلص المائى للثوم على كلا النوعين من الأكاروس تحت الظروف النصف حقلية و ثبت تأثيره على كلا النوعين و لكنالحلم القرمزى كان أكثر مقاومه حيث سجلت النتائج نسبه خفض فى التعداد بنسبة ٨٠.١٤% و ارتفعت هذه النسبة الى ٨٩.٧٥% فى العنكبوت ذو البقعتين.