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In vitro Comparative Efficacy of Various Concentrations of Aqueous and Methanolic Leaf Extract of *Ocimum sanctum* against *Tetranychus urticae* Koch in Tomato

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A B S T R A C T

In present investigation, the bioefficacy of aqueous and methanolic leaf extracts of *Ocimum sanctum* were tested to evaluate their toxic effects at different concentration (10.00, 7.50, 5.00, 2.50, 2.00, 1.50, 1.00 and 0.50 percent) against mixed population of *Tetranychus urticae* on tomato during 2018 under *in vitro* conditions. *T. urticae* responded to the mite in a concentration dependent manner i.e. lowest number of live mites and highest mortality in population was obtained with highest concentration tested (10.0%). Out of an initial number 10 mite, significantly less number of mites were recorded at 10.00 percent concentration of *O. sanctum* leaf extract followed by 7.50, 5.00, 2.50 and 2.50 per cent concentration than 1.50, 1.00 and 0.50 percent treatment and control. Direct spray bioassay results clearly revealed that aqueous and methanolic leaf extract of *O. sanctum* possessed acaricidal activity. Mites responded to extract in a concentration dependent manner. The methanolic leaf extract was more potent to *T. urticae* causing 20 to 71.9 percent mortality at 0.5 to 10 percent concentration as compared to aqueous leaf extract (6.7 to 61% mortality). The LC_{50} value in methanol extract was 3.46 percent while it was 7.06 percent in aqueous extract against *T. urticae*.

Introduction

Tomato offer food, shelter and reproductive sites (Mehta, 2012) to more than 200 insects and mites worldwide (Lange and Bronson, 1981). Two spotted spider mite, *T. urticae* (Acari: Tetranychidae) is an important agricultural pest of solanaceous crops including tomato in greenhouses and open fields worldwide (Awad *et al.*, 2018). Apart from tomato, it is known to attack about 1200 species of plants, out of which more than 150 are economically important such as corn, cotton (Aucejo *et al.*, 2003), okra (Geroh, 2011), cucumber (Kanika *et al.*, 2013), eggplant (Sonika *et al.*, 2017), peppers and beans (Uddin *et al.*, 2015). It causes 70 percent damage depending on period of

infestation on several commercial crops including peanuts, soybean, and others (Boubou *et al.,* 2011). Under heavy infestations of *T. urticae,* tomato plants suffer massive leaf drop and yield loss. Because of the negative impact of *T. urticae* on tomato crops, various management practices are followed against mites. There are several reports on botanicals like, neem (Martinez-Villar *et al.,* 2005), common tancy, wormwood extracts (Chiasson *et al.,* 2001), aak, oleander (Islam *et al.,* 2008), marigold, garlic, pepper (Boyd and Alverson, 2000; Rusch, *et al.,* 2010), which are effective against *T. urticae* adults.

Tulsi (Holy Basil), *Ocimum* genus is a traditional plant considered sacred by the Hindus. It has small stems and leaves with an appreciable aroma due to essential oils, such as eugenol, methyl-chavicol, methyl-eugenol, saffron, geranial, thymol and linalool (Deshpande *et al.,* 1997; Blank *et al.,* 2005). Its use as an insecticide, nematicide, fungicide and antimicrobial compound has been reported (Mishra and Mishra, 2011). Leaf extracts of *O. sanctum* caused 100 and 75 per cent mortality in mushroom mite, *Luciaphorus* sp. (Bussaman *et al.,* 2012) and *T. urticae* (Kanniammal and Chinniah, 2012), respectively. It showed 65 per cent repellency against *Petrobia harti*, another tetranychid mite (Mitra *et al.,* 2015).

Water extract of *O. basicilum* (@15%) caused 74 percent adult mortality and complete inhibition of *T. urticae* fecundity (Isabel *et al.,* 2016). Ogayo *et al.,* (2019) reported that plant extract of *O. gratissimum* and *Leonotise nepetifolia* at 12 percent concentration showed highest efficacy (82.75 and 69.06 %) against *T. urticae* which led to increase in pod numbers and pod yield in French bean. Keeping these views in mind, the present study was made to study the effect of aqueous and methanolic extracts of leaves of tulsi (*Ocimum sactum*) for management of this pest of tomato.

Materials and Methods

Under *in vitro* conditions, bio-efficacy of aqueous and methanolic leaf extracts of *Ocimum sanctum* were evaluated against *T. urticae* to determine their acaricidal activity against *T. urticae* under standardized conditions (30±10C, 80-85% RH) in the Acarology Lab, Department of Zoology, CCS HAU during, 2018. Infested leaves were plucked from the field crop and brought to the laboratory. Under stereo zoom microscope, mobile stages of mite were picked with the help of bird's feather pick and released on the separate untreated leaf. Each subset was replicated three times containing 10 mites in each replicate.

Efficacy of Ocimum sanctum against Tetranychus urticae

Aqueous and Methanolic Leaf extract *Tulsi* (*Ocimum sanctum*) were evaluated for bioeffficacy studies against *T. urticae* using Leaf Disc Technique. In this technique, a moist cotton bed was prepared in a petri plate with covering lid. The bed was circled with moist cotton pad to prevent the escape of mites. This leaf was kept above the moist cotton pad in the centre of the petri plate. The extracts were sprayed directly on tomato leaves after releasing the mites on them and daily number of mites was counted.

Preparation of aqueous and methanolic leaf extract of *Ocimum sanctum*

Coarsely ground leaves (250g) of *O. sanctum* were soaked in 250 litre of distilled water for 48 hours at room temperature. Intermittent shaking was done during this period. Later, the solution was filtered through the muslin cloth and stored in glass bottle for use in

experimentation. This stock was considered as 100 percent and further dilutions were made by adding distilled water to obtain 10, 7.5, 5, 2.5, 2, 1.5, 1 and 0.5 percent concentrations.

Methanolic extract of leaves were prepared following the standard procedure of refluxing and distillation (Kumar *et al.,* 2001). Leaves were cleaned and were allowed to shade-dry for a month, after which they were crushed into bits. Crushed leaves (250 g) were taken in a round bottom flask of 2 litre capacity and methanol AR grade was added to it to immerse the bits and kept it for overnight. Refluxing was done by fitting the flask with a water condenser and boiling the set using a heating mantle for 8h. The extract was then filtered out of the flask and concentrated by distillation process. This procedure of refluxing and distillation was repeated thrice for complete extraction of active ingredients from leaves. In this process 300 to 250 ml of methanolic extracts were obtained respectively. This stock was considered as 100 percent and further dilutions were made by adding methanol to obtain different concentrations *viz.,* 10.00, 7.50, 5.00, 2.50, 2.00, 1.50, 1.00 and 0.50 percent of methanolic extracts. Suitable control without water and with water was maintained for the experiments.

Direct spray technique

The leaves from untreated and uninfested healthy plants were used in these experiments. Under stereo zoom microscope, mobile stages of mites were picked with the help of bird's feather pick and released on the separate untreated leaf disc. Before releasing the mites, leaves were washed properly. Separate sets were maintained for aqueous and methanolic leaf extract. Each set contained 8 subsets of different concentrations (treatment) and two controls. Similarly, subsets of different concentrations were prepared for methanolic extracts. Each subset was replicated three times containing 10 mites per replicate. Each concentration was sprayed separately on leaf with the help of hand automizer. The leaf was placed on moist cotton bed of the covered petri plate.

Observations on the live mite stages were recorded after every 24 h under microscope in each treatment. These were compared with control. Before considering the mite stage as dead, it was probed lightly with the help of bird's feather pick to detect any movement. Observations were continued after every 24 h till the appearance of the next stage or mortality of test stage. The observed mortality was converted into percent mortality (% reduction) and corrected mortality was obtained after deducting the mortality in control treatments. The data were used for calculating the LC_{50} of both the extracts.

The percent reduction in mite count as compared to pre- treatment count was calculated by the formula:

$$\text{Percent Reduction} = \frac{\text{(Pre-treatment count - Number of live mites after treatment)}}{\text{Pre- treatment count}} \times 100$$

Results and Discussion

Statistical analysis

For assessing the effectiveness of the treatments, mean numbers of *T. urticae* were pooled and analyzed statistically. Critical difference (CD) was calculated between the treatments to see the impact of population buildup of *T. urticae* on tomato by single and factorial CRD (*in vitro*) method. Data for evaluating the effect of *O. sanctum* against *T.*

urticae under *in vitro* conditions was subjected to two factorial CRD. Data transformation was applied wherever necessary. CD was calculated in each case and means of treatments were compared to see the significant difference between the treatments and with control at different observation periods. CD was also used to find out the most effective extract and its concentration.

Bioefficacy of aqueous and methanolic extract of *Ocimum sanctum* leaves were evaluated at different concentrations against mixed population of *T. urticae* in tomato during 2018 under *in vitro* and *in vivo* conditions. The results revealed that all the treatments possessed acaricidal activity against *T. urticae* but to different extents. The obtained results have been summarized in Tables and figures are described below:

Aqueous leaf extracts of *Ocimum sanctum*

Direct spray bioassay results clearly revealed that aqueous leaf extract of *O. sanctum* possessed acaricidal activity. Mites responded to extract in a concentration dependent manner i.e. lowest number (4.29 mites/sq cm leaflet) of live mites was obtained with highest concentration (10%) and highest number (9.33 mites/sq cm leaflet) with lowest concentration (0.5%) (Table 1). All the treatments were significantly better than controls (9.67, 9.43 mites/sq cm leaflet) (CD=0.08; p=0.05). Both the control treatments, water sprayed and unsprayed leaves along with the lower two *O. sanctum* aqueous extract treatments (9.29, 9.33 mites/sq cm leaflet) were statistically comparable with each other. This suggested that water treatment did not cause significant mortality in mites. The treatments, 5.0 (6.57 mites/sq cm leaflet) and 2.5 (6.86 mites/sq cm leaflet) per cent, 2.0 (7.52 mites/sq cm leaflet) and 1.5 (8.00 mites/sq cm leaflet) per cent were statistically comparable in terms of mean number of mites. Statistical analysis revealed a significant effect of observation period. The number of mites significantly decreased to 9.40 after first day of spray as compared to initial 10 mites (CD=0.07; p=0.05). After 2nd and 3rd day of spray, the number of mites decreased to 9.07 and 8.67 mites; showing non significant decrease with each other.

Thereafter, significantly lower numbers of mites (8.10, 7.43, 6.07 and 4.30) were recorded after 4, 5, 6 and 7 day of spray. The ANOVA revealed significant interaction between the treatment and observation periods (CD=0.22; p=0.05) (Table 1). This showed that at each observation period, higher concentrations were more effective in reducing the *T. urticae* population than lower concentrations.

Methanolic leaf extracts of *Ocimum sanctum*

Similar results were obtained with methanolic leaf extract of *O. sanctum* showing significantly lower number of mites as compared with control (9.29, 9.81 mites/sq cm leaflet in water spray and unsprayed leaf disc, respectively) (CD=0.09; p=0.05). Both the control treatment did not cause any significant mortality in *T. urticae* and were at par with each other. Among the treatments, higher reductions (2.81, 3.57 mites/sq cm leaflet) were witnessed at higher concentrations (10, 7.5%).

The number of mites was 5.14, 5.48, 6.00, 6.76, 6.91 and 8.00 mites/sq cm leaflet at 5.0, 2.5, 2.0, 1.5, 1.0 and 0.5 percent of methanolic extract (Table 2). The concentrations, 5 and 2.5, 1.5 and 1.0 per cent were at par with each other. A significant effect of observation period was recorded in this bioassay. First day after the spray,

number of mites significantly decreased to 8.37 as compared to initial 10 mites (CD=0.08; p=0.05). Thereafter it showed a significant gradual decrease to 7.93, 7.43, 6.47, 5.73, 4.87 and 3.83 after 2nd, 3rd, 4th, 5th, 6th and 7th day spray. The number of mites recorded after 1st and 2nd day was statistically comparable. Likewise, the numbers of mites observed after 2nd and 3rd day of spray do not differ significantly with each other. Interaction between the treatment and observation periods was found significant (CD=0.25; p=0.05) (Table 2). This showed that at the end of study period (7th day), lower *T. urticae* number was observed at higher concentrations than at lower concentration.

Table.1 Estimation of vitamin B-6 (ppm) in Areca nut collected from different locations of Karnataka

Concentration (%)	Pre treatment count	Number of live mites/leaf disc							Mean
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
10.0	10.00	7.67 (2.94)	7.00 (2.82)	6.33 (2.70)	4.33 (2.31)	3.00 (1.99)	1.33 (1.49)	0.33 (1.14)	**4.29 (2.20)**
7.5	10.00	8.33 (3.05)	7.67 (2.94)	6.33 (2.71)	5.33 (2.52)	4.33 (2.31)	1.67 (1.63)	0.00 (1.00)	**4.81 (2.31)**
5.0	10.00	9.00 (3.16)	8.67 (3.11)	8.33 (3.05)	7.67 (2.94)	7.00 (2.83)	4.67 (2.38)	0.67 (1.24)	**6.57 (2.67)^{a}**
2.5	10.00	9.33 (3.21)	9.33 (3.21)	9.00 (3.16)	7.67 (2.94)	6.67 (2.76)	4.33 (2.31)	1.67 (1.63)	**6.86 (2.75)^{a}**
2.0	10.00	10.00 (3.32)	9.33 (3.21)	8.67 (3.11)	8.33 (3.05)	7.67 (2.94)	6.33 (2.70)	2.33 (1.82)	**7.52 (2.88)^{b}**
1.5	10.00	10.00 (3.32)	9.67 (3.27)	9.00 (3.16)	8.67 (3.11)	8.00 (3.00)	6.33 (2.71)	4.33 (2.31)	**8.00 (2.98)^{b}**
1.0	10.00	9.67 (3.27)	9.67 (3.27)	9.67 (3.27)	9.67 (3.27)	9.33 (3.21)	9.00 (3.16)	8.00 (3.00)	**9.29 (3.21)^{c}**
0.5	10.00	10.00 (3.32)	9.67 (3.27)	9.67 (3.27)	9.67 (3.27)	9.33 (3.21)	9.00 (3.16)	8.00 (3.00)	**9.33 (3.21)^{c}**
Control (with water)	10.00	10.00 (3.32)	9.67 (3.27)	9.67 (3.27)	9.67 (3.27)	9.33 (3.21)	9.00 (3.16)	8.67 (3.11)	**9.43 (3.23)^{c}**
Control (Unsprayed)	10.00	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.67 (3.27)	9.00 (3.16)	9.00 (3.16)	**9.67 (3.27)^{c}**
Mean	10.00	**9.40 (3.22)^{b}**	**9.07 (3.17)^{a,b}**	**8.67 (3.10)^{a}**	**8.10 (3.00)**	**7.43 (2.87)**	**6.07 (2.59)**	**4.30 (2.14)**	

Figures in parentheses are √n +1 transformation
C.D. for Treatment (T) = (0.08), SE(m) =(0.03);
C.D. for Observation Period (OP) =(0.07), SE(m) =(0.03)
C.D. for Interaction OP × T= (0.22), SE(m) =(0.08);
Values with the same superscript do not differ significantly

Table.2 *In vitro* bioassay of *Ocimum sanctum* methanolic leaf extract against *Tetranychus urticae*

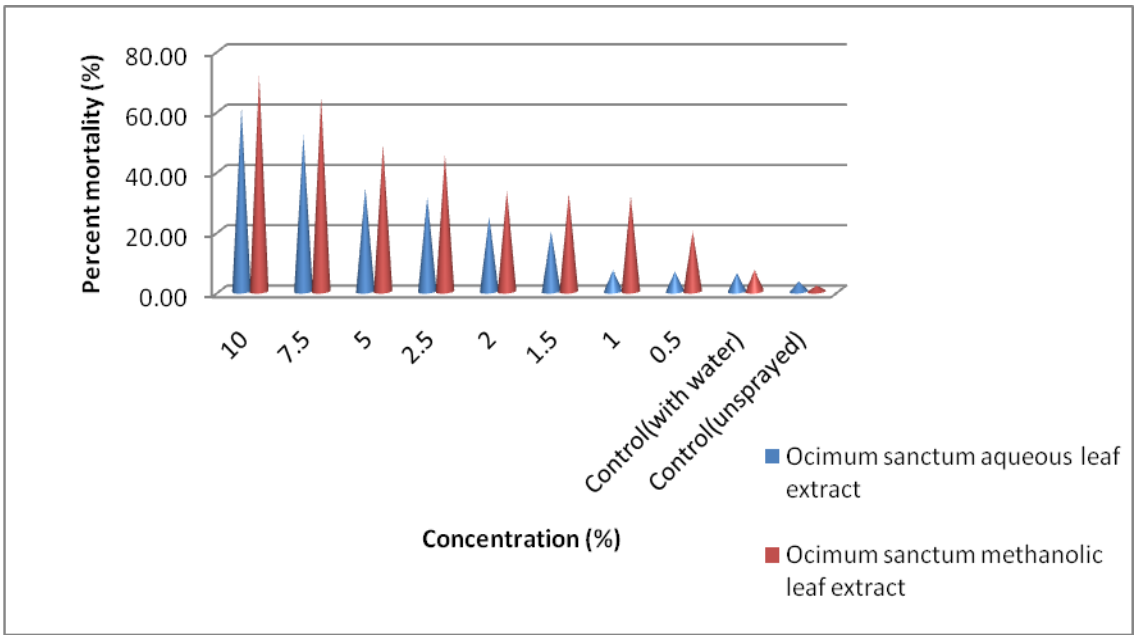
Concentration (%)	Pretreatment count	Number of live mites/leaf disc							Mean
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
10.0	10.00	7.00 (2.83)	5.67 (2.58)	4.00 (2.23)	2.33 (1.82)	0.67 (1.28)	0.00 (1.00)	0.00 (1.00)	**2.81 (1.82)**
7.5	10.00	7.67 (2.94)	6.33 (2.71)	5.67 (2.58)	3.33 (2.06)	1.67 (1.63)	0.33 (1.14)	0.00 (1.00)	**3.57 (2.01)**
5.0	10.00	8.00 (3.00)	7.67 (2.94)	7.00 (2.83)	5.00 (2.44)	4.67 (2.38)	2.67 (1.91)	1.00 (1.38)	**5.14 (2.41)^{a}**
2.5	10.00	7.67 (2.94)	7.33 (2.89)	6.67 (2.76)	6.33 (2.71)	5.00 (2.44)	3.67 (2.14)	1.67 (1.63)	**5.48 (2.50)^{a}**
2.0	10.00	8.00 (3.00)	7.67 (2.94)	7.33 (2.89)	6.00 (2.62)	6.00 (2.62)	4.67 (2.38)	2.33 (1.82)	**6.00 (2.62)**
1.5	10.00	8.33 (3.05)	8.33 (3.05)	7.67 (2.94)	7.33 (2.89)	6.67 (2.76)	5.67 (2.58)	3.33 (2.08)	**6.76 (2.77)^{b}**
1.0	10.00	8.67 (3.11)	8.33 (3.05)	8.00 (3.00)	6.67 (2.76)	6.33 (2.71)	5.67 (2.58)	4.67 (2.38)	**6.91 (2.80)^{b}**
0.5	10.00	9.00 (3.16)	8.67 (3.11)	8.67 (3.11)	8.33 (3.05)	7.33 (2.89)	7.00 (2.83)	7.00 (2.83)	**8.00 (3.00)**
Control(with water)	10.00	9.33 (3.21)	9.33 (3.21)	9.33 (3.21)	9.33 (3.21)	9.33 (3.21)	9.33 (3.21)	9.00 (3.16)	**9.29 (3.21)^{c}**
Control(unsprayed)	10.00	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	10.00 (3.32)	9.67 (3.27)	9.67 (3.27)	9.33 (3.21)	**9.81 (3.29)^{c}**
Mean	10.00	**8.37 (3.06)^{a}**	**7.93 (2.98)^{a,b}**	**7.43 (2.89)^{a}**	**6.47 (2.69)**	**5.73 (2.52)**	**4.87 (2.30)**	**3.83 (2.05)**	

Figures in parentheses are √n +1 transformation
C.D. for Treatment (T) = (0.09), SE(m) =(0.03);
C.D. for Observation Period (OP) =(0.08), SE(m) =(0.03)
C.D. for Interaction OP × T= (0.25), SE(m) =(0.08);
Values with the same superscript do not differ significantly

Table.3 LC_{50} of *Ocimum sanctum* extract for *Tetranychus urticae*

Ocimum sanctum leaf extract	Direct spray bioassay (Leaf disc method)					
	n	Slope	Intercept	LC_{50} (%)	$\chi 2$	Df
Aqueous extract	10	1.25	3.93	7.06	12.59	5
Methanolic extract	10	1.22	4.29	3.46	12.59	5

Fig.1 Comparative efficacy of aqueous and methanolic leaf extract of *Ocimum sanctum* against *Tetranychus urticae*



An analysis through graphical representation showed the comparative efficacy of aqueous and methanolic extracts of *O. sanctum* against *T. urticae* at different concentrations (Fig. 1). The higher mite mortality in methanolic leaf extract showed that it is more potent to *T. urticae* than aqueous leaf extract causing 71.9 to 20 percent mortality at 10 to 0.5 percent concentration as compared to 61 to 6.7 percent mortality in aqueous extract.

Determination of concentration-mortality response (LC_{50})

The LC_{50} values (concentration at which 50 percent mortality occur in *T. urticae* population) along with regression statistics under direct spray bioassay were calculated using standard probit analysis method and are presented in Table 3 for both extracts of *O. sanctum*. The LC_{50} value in methanol extract was 3.46 percent while it was 7.06 percent in aqueous extract against *T. urticae*. The value of slope (1.25 and 1.22) was less than for both the extracts. This showed that further increase in concentrations will lead to significant decrease in number of *T. urticae.* The intercept value was 3.93 and 4.29, respectively in both the extract. The present study showed that methanolic extract was more effective against *T. urticae* than aqueous extract because of lower LC_{50} value.

Tulsi (*Ocimum sanctum*) is a traditional plant which possesses important nematicide, fungicide and insecticidal properties (Deshpande *et al.,* 1997; Nanasombat and Lohasupthawee, 2005; Mishra and Mishra 2011). Phenolic of botanicals compounds play an important role in herbivore-host plant interaction; plants with a high concentration of phenols are often less attractive hosts for many herbivorous insects and mites than plants with a low content of these secondary metabolites (Gogoi *et al.,* 2001; Sahayaraj *et al.,* 2003). Secondary metabolites present in plants with insecticidal effects may act as inhibitors of insect feeding or hinder growth, development, and reproduction (Spochacz *et al.,* 2018).

In present study aqueous and methanolic extract of *O. sanctum* were used for control of *T. urticae*. Mites responded to *O. sanctum* aqueous and methanolic leaf extracts in a concentration dependent manner in present study i.e. lowest number (4.29 mites/sq cm leaflet) of live mites was obtained with highest concentration (10%) and highest number (9.33 mites/sq cm leaflet) with lowest concentration (0.5%). The present study showed that methanolic extract (3.46 percent) was more effective against *T. urticae* than aqueous extract (7.06 percent) because of lower LC_{50} value. Methanolic extracts *Ocimum tenuiflorum* (3%) exhibited acaricidal activities against *Tetranychus neocaledonicus* and resulted in the 82.2 percent (Chayengia *et al.,* 2010) and 93.3 percent mortality at (Roy *et al.,* 2011) mortality. Kanniammal and chinniah (2012) also reported that *O. sanctum* caused highest mortality of *T. urticae* (75.07%). Isabel *et al.,* (2016) worked on nine Brazilian accessions. They found that, one basil accession, OVRS showed high toxicity against *T. urticae* females at 15 percent concentration water-extract; the mortality rate was 75percent and complete inhibition of fecundity was found on peanut. *Ocimum* sp. also showed acaricidal effects on several parasite mites. Kamaraj *et al.,* (2008) *Ocimum gratissimum* L. oil and its constituents have fumigant and repellent activity against storage pests and mites (Kim *et al.,* 2003; Ogendo *et al.,* 2008) as well as malaria vectors *Aedes aegypti* L. (Diptera: Culicidae) and *Culex quinquefasciatus* Say (Diptera). The essential oils of *Ocimum basilicum* Linn., *Coriandrum sativum* Linn., *Eucalyptus globulus* Labill, *Mentha piperita* Linn. and *Satureja hortensis* Linn. were toxic to poultry red mite [*Dermanyssus gallinae* (De Geer)], and, when using the *in vitro* direct contact method, these essential oils at the dose of 0.6mg/cm could result in mortality rates over 80 percent after 24 h of contract (Magdas *et al.,* 2010). Manzoor *et al.,* (2011) reported that *O.sanctum* oil as toxicant and repellent agent against termite, *Heterotermes indicola*

In conclusion, the present study focused upon management practices against *T. urticae* in tomato. 10.0% concentration of both extract was most potent against *T. urticae.* More studies are required in this area and *O. sanctum* leaf extract can be used for protection of tomato crops against *T. urticae.*

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