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Study of potential activity of clove oil 10 % emulsifiable concentrate formulation on Two-spotted spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract
Clove oil is reported to have a great range of biological activities against many species of pests; termites, cockroaches, aphids, weevils and moths, and many different usages such as perfume and food flavoring agent. This study aimed to determine the acaricidal activity of clove oil emulsifiable concentrate new formulation against two-spotted spider mites *Tetranychus urticae*. Clove oil was formulated as 10 % emulsifiable concentrate (EC). The new formula passed successfully all physical and chemical tests reported for emulsifiable concentrates. It was then tested biologically on the individuals of two-spotted spider mite *T. urticae* Koch (Acari: Tetranychidae) on two host plants beans and squash under laboratory conditions. In both cases, there were a direct relationship between the increase in the concentration of the formulation and the percentage of inhibition on the individuals of the two-spotted spider mite, after 72 hours of treatment, at concentrations of 20, 40, and 80 mg/ml the new formulation showed 10.30, 44.8, and 75 percent inhibition, respectively in case of beans, while squash at the same concentrations and for the same period of treatment showed 33.3, 40.7, and 51.9 percent inhibition, but the effect in case of beans was greater than that in case of squash as its EC_{50} value was lower than that in case of squash. In the case of the former, it was 39.81 mg/ml, whereas in the case of the latter, it was 79.43 mg/ml. Therefore, the new clove oil formulation can be used to combat the two-spotted spider mite.

Keywords: Clove oil, Emulsifiable Concentrate Formulation, Spider mite, *Tetranychus urticae*

INTRODUCTION

Clove oil is derived from the clove plant *Syzygium aromaticum,* and is an essential oil. Its insecticidal and repellant properties against a variety of pests have been extensively researched (Chaieb *et al.* 2007, Kafle and Shih 2013, Corte´s-Rojas *et al.*, 2014), including Asian citrus psyllid (Mann *et al.* 2010); termites (Pandey *et al.* 2012); fire ants (Appel *et al.* 2004); aphids (Kareem *et al.* 2012); cockroaches (Sharawi *et al.* 2013); moths (Birah *et al.* 2010) and weevils (Mishra *et al.* 2013). It also acts as a larvicide, pupicide, adulticide, oviposition deterrent, and ovicide against *Aedes aegypti*, *A. albopictus*, and *Culex quinquefasciatus* mosquito vectors (Phasomkusolsil and Soonwera 2012; Shapiro 2011; Bhat and Kempraj 2009). Clove oil is also commonly used as a fragrance and food flavouring additive. It possesses a wide range of pharmacological effects, including antibacterial, anti-inflammatory, antioxidant, analgesic and anticancer properties (Kamatou *et al.* 2012).

Archita *et al.,* (2015) investigated the efficacy of clove oil on the red spider mite, *Oligonychus coffeae* Nietner (Acari: Tetranychidae), which infests tea plants. They found that *O. coffeae* mortality varied depending on the concentrations and duration of the mites' exposure time after application of the oil. Furthermore, clove oil at specific concentrations has been shown to be effective against adult mites.

Two-spotted spider mite (TSSM), *Tetranychus urticae*

Koch, is a frequent pest of many crops (Adeney *et al.* 2009). TSSM infest stems, leaves, and branches, causing varying degrees of damage. They often feed on the leaves, damaging the epidermis and causing stippling, blotching, or bronzing, reducing yield qualitatively and quantitatively. (Abdallah *et al.,* 2009 and Faris *et al.,* 2004). It also develops silk webbing, which is apparent at high infestation levels (Alzoubi and Cobanoglu, 2006), which limits the plant's ability to manufacture carbohydrates and, as a result, lowers total output (Fouly *et al.,* 2019)

Acaricide resistance in phytophagous mites is a regularly rising problem, particularly in *T. urticae* and other Tetranychids (Van Leeuwen *et al.* 2009). Many populations of the two-spotted spider mite have gained global resistance to a variety of novel acaricides and insecticides during the 1990s (Arthropod Pesticide Resistance Database, http:// www.pesticideresistance.org/). Furthermore, after only a few years of use, two-spotted spider mite populations have evolved a significant level of resistance to newly introduced compounds, with cross-resistance reported to other compounds with the same mode of action.–There have been reports of *T. urticae* developing resistance to pesticides in general and acaricides in particular, such as fenazaquin (Vassiliou and Kitsis, 2013), propargite (Kumari *et al.,* 2015), bifenazate (Van Leeuwen *et al.,* 2006), and spiromesifen (Sato *et al.,* 2016).

An active ingredient is usually combined with various inert components known as additives or adjuvants in a pesticide formulation. The primary goal of additions is to enhance the active ingredient's effectiveness. Spreaders, wetting agents, stickers, foaming agents, and compatibility agents are all common additives (Libs and Salim, 2017).

The objective of this work was to study the acaricidal activity of clove oil emulsifiable concentrate formulation against the two-spotted spider mites *T. urticae* as a new step in the completion of our target program to obtain safe, eco-friendly active ingredients and formulating it in the form of local formulations for the control of spider mites.

MATERIALS AND METHODS

Tested chemicals

a) Clove oil: Supplied by Agricultural Development Markets, Nadi El Seid St., Dokki, Giza, b) Surface active agents: Supplied by EL-Gomhoria Co., Cairo, Egypt.

Physico-chemical properties of the new formulation basic components:

Active ingredients

a) Solubility: At 20°C, the total solubility or miscibility of one gram of active ingredient was tested by measuring the volume of distilled water, ethanol, acetone, xylene, and DMF (Nelson and Fiero, 1954). The following equation was used to compute the percent solubility:

% solubility = W/V x 100 ………Eq. 1

Where; W= active ingredient weight, V= volume of solvent required for complete solubility.

b) Free acidity or alkalinity: It was evaluated by using the procedure outlined in World Health Organization guidelines (1979).

Surface active agents

a) Surface tension: It was calculated by using Du-Nouy tensiometer for solutions containing 0.5 % (W/V) surface active agent according to American Society of Testing Materials (ASTMD-1331) (2001).

b) Hydrophilic-lipophilic balance (HLB): A surfactant's solubility in water was used as a useful approximation to its hydrophilic-lipophilic balance (Lynch and Griffin, 1974).

c) Critical micelle concentration (CMC): The concentration of the tested surfactants at which the surface tension of the solution does not decrease as the surfactant concentration increases (CMC), was calculated using the method described by Osipow,(1964).

d) Free acidity or alkalinity: It was determined as previously stated.

Preparation of clove oil as emulsifiable concentrate (EC) formulation

The novel formula was made using the procedure Soliman (2005) outlined, which involved dissolving clove oil in a sufficient amount of solvent, adding the emulsifying agent, and stirring the solution for 1 hour. The solution was then diluted to 100 mL using the same solvent used for dissolution, violently agitated to ensure homogeneity, filtered out, and preserved in a tightly sealed vial, and the following physico-chemical parameters for emulsifiable concentrate formulation were determined:

a) Emulsion stability test: It was performed according to FAO/WHO MT 36.3. (2010).

b) Accelerated storage: It was evaluated according to Collaborative International Pesticides Analytical Council (CIPAC M46.1. (1995).

Spray solution at the field dilution rate

The following physico-chemical properties for the spray solution were determined:

a) Surface tension: It was performed as mentioned before.

b) Viscosity: Brookfield viscometer model DVII+Pro was used to determine viscosity, with centipoise being the unit of measurement according to American Society of Testing Materials (ASTM D-2196) (2005).

c) Electrical Conductivity: It was measured with Cole-Parmer PH/Conductivity meter 1484-44, where μmhos is the unit of electrical conductivity measurements according to (Dobrat and Martijn, 1995).

d) pH: It was evaluated by using Cole-Parmer pH conductivity meter 1484-44 according to (Dobrat and Martijn, 1995).

Bioassay

Efficacy on individuals under laboratory conditions

Acaricidal effect of clove oil emulsifiable concentrate formulation on the individuals of the two-spotted spider mites of *T. Urticae* were carried out according to the leaf disk assay conducted according to the method described by Pree *et al.,* (1989). The *Acalypha marginata* leaf disks (2.5 cm in diameter) were dipped in series concentrations of the tested formulation (20000, 40000 and 80000 ppm). Concentration mortality relation was based on tests with three concentrations of the formulation, and was expressed as mg/ml of the active ingredient. The discs were put on wet cotton wool in petri-dish and kept under constant conditions (25 ± 2 ˚C, 65 ± 5 % relative humidity (RH) and 16:8 photoperiod. Ten adult females (one day old) of *T. urticae* were transferred on each disc with a fine brush. Control discs were dipped in distilled water. Tests were repeated 3 replicates for each concentration of each formulation. In addition to the control, the number of live and dead mites was counted 24, 48, 72, 144, and 168 hours after treatment.

Statistical analysis

Percentages of mortality were corrected according to Abbott's formula (1925) as follows

Mortality % = The died number of TSSM counted after treatment / The died number of TSSM counted before treatment x 100 ………Eq. 2

and the concentration inhibition regression lines were calculated through the relationship between concentration logarithm and probit percentage mortality, according to the method of Finney (1952) and the toxicity was calculated according to the equation

Toxicity index = EC_{50} of the most effective compound / EC_{50} of the tested compound x 100 ………Eq. 3

RESULTS AND DISCUSSION

Table 1 shows the physico-chemical properties of clove oil. It showed complete solubility in acetone (100 %) and medium solubility in water and xylene (50 %), showing alkaline properties (free alkalinity as sodium hydroxide). The type of formulation limited by the pesticide solubility and its hydrolytic properties (FAO/ WHO Meeting (2002), solubility and free alkalinity results showed that the suitable formulation type for this active ingredient was emulsifiable concentrate.

Clove oil local 10 % emulsifiable concentrate formulation tested for the physical and chemical properties under normal and accelerated storage conditions showed the same value of free alkalinity, in addition, no changes were observed for spontaneity and emulsion stability under both normal and accelerated storage conditions. The new formula demonstrated its ability to retain its physical and chemical properties under both storage conditions (Table 2).

The physico-chemical properties of the spray solution are shown in Table 3. These properties determine to a large extent how far the new formula is effective. The spray solution showed lower surface tension 40.8 dyne/cm compared to that of water 72 dyne/cm of water. According to (Pereira *et al.,* 2016) , the decrease of surface tension of pesticide spray solution gives a

Table 1. Physico-chemical properties of clove oil as an active ingredient

Solubility % (W/V)			Free alkalinity as NaOH
Water	Acetone	Xylene	
50	100	50	8.35

Table 2. Physico-chemical properties of clove oil 10 % emulsifiable concentrate formulation before and after accelerated storage.

Before storage							After storage						
Spontaneity %		Emulsion stability		Foam		Free alkalinity as NaOH	Spontaneity %		Emulsion stability		Foam		Free alkalinity as NaOH
Hard	Soft	Hard	Soft	Hard	Soft		Hard	Soft	Hard	Soft	Hard	Soft	
100	100	pass	Pass	-	-	0.04	100	100	Pass	pass	-	-	0.04

Table 3. Physico-chemical properties of clove oil EC (emulsifiable concentrate) spray solution with 0.5 % concentration.

Surface tension dyne/cm	Viscosity cm/poise	Electrical conductivity μ mhos	pH
40.8	10.4	56	7.8

prediction of increasing wettability and spreading on the treated surface, resulting in increasing pesticidal efficiency. Also the increase in viscosity could increase the pesticidal efficacy as stated by (Spanoghe *et al.,* 2007) who reported that increasing viscosity of spray solution cause reduction drift and increase the retention sticking and insecticidal efficiency. Electrical conductivity also affected the efficiency of the pesticide greatly, as the increase in electrical conductivity could increase the biological efficacy of the pesticide as decided by (El-Sisi *et al.,* 2011), as they reported that the increase in electrical conductivity of the spray solution would lead to deionization of insecticides and increase its deposit and penetration in the tested surface with a consequence increase in the insecticidal efficiency.

Three successive serial concentrations from clove oil new emulsifiable concentrate tested on (TSSM) individuals on squash as host plant under laboratory conditions are shown in Table 4. A concentration of 20 mg/ml inhibited individuals by 3.3, 10.3 and 33.0 %. 40 mg/ml inhibited individuals by 10.0, 27.6 and 40.7 % whereas 80 mg/ml showed 26.7, 37.9 and 51.9 % inhibition after 24, 48 and 72 hrs. from treatment respectively. It was clear that there was a gradual uniform increase in the percentage of inhibition for each concentration with the increase in exposure periods.

Table 5 shows the effect of clove oil EC on the individuals of (TSSM) on beans as a host plant by the three previously mentioned serial concentrations under laboratory conditions. The increase in exposure periods for all tested concentrations resulted in a gradual increase in the percentage of inhibition as in the case of squash, the higher the concentration, the higher the percentage of inhibition (80 mg/ml) showed the highest effect between all tested concentrations after 168 hrs. from exposure. The results could be explained on the basis of the increase of the active ingredient concentration that reaches the active site with the increase of exposure periods, which may also be attributed to the role of surface-active agents (additives) used in the formulation in facilitating the penetration of the pesticide (Fishel, 2010).

LCP lines for the experimentation of clove oil 10 % EC on (TSSM) individuals on squash and beans as host plants under laboratory conditions are represented in Table 6. EC_{50} and toxicity index showed that the effect

Table 4. Effect of clove oil EC % on the individuals of (TSSM), *Tetranychus urticae* on squash as a host plant under laboratory conditions

Concentration (mg/ml) / Exposure periods hrs.	% of inhibition at		
	20	**40**	**80**
24	3.3	10	26.7
48	10.3	27.6	37.9
72	33.3	40.7	51.9

Table 5. Effect of clove oil EC % on the individuals of (TSSM), *Tetranychus urticae* on beans as a host plant under laboratory conditions

Concentration (mg/ml) / Exposure periods hrs.	% of inhibition at		
	20 mg/ml	**40 mg/ml**	**80 mg/ml**
24	00.0	20	40
48	3.3	36.7	55.7
72	10.3	44.8	75
144	17.2	55.2	79.3
168	40.9	77.3	100

Table 6. LCP lines of clove oil EC % on the individuals of (TSSM), *Tetranychus urticae* on both host plants under laboratory conditions.

Local Formulation	**Host plant**	**EC_{50} mg/ml**	**Slope**	**Toxicity index**
Clove oil EC	Beans	39.810	0.8	100
	Squash	79.432	0.8	158

EC: Emulsifiable concentrate

of clove oil new formula was higher in the case of beans than in the case of squash, the effect that may be attributed to the nutrient components of the host species, but the slope value was the same in both cases indicating the same mode of action of the new formulation on (TSSM) individuals for beans and squash. Agremic 8.4 % SC recommended as acaricide containaing synthesized active ingredients abamectin showed LC_{50} 0.0000102 mg/ml against *T. urticae* (Mohammed *et al.,* 2018). Although the recommended acaricide is more effective than clove oil formulation, it has a number of drawbacks, including environmental contamination and resistance as a result of repeated use, whereas clove oil formulation is environmentally friendly because it is made from plant material in addition to the lack of resistance.

Conclusion

Clove oil was formulated as 10 % emulsifiable concentrate and passed all reported physico-chemical properties. The new formula was tested on the individuals of the two-spotted spider mites *T. urticae* on beans and squash as host plants under laboratory conditions. It showed a good inhibition effect on the individuals on both host plants, although the effect was greater in the case of beans than in the case of squash. The significance of this research stems from the formulation of clove oil as an emulsifiable concentrate, which is a safer alternative to standard synthetic pesticide formulations. As a result, it is suggested that the new clove oil formulation can be used to combat TSSM.

Conflict of interest

The authors declare that they have no conflict of interest.

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