

Effect of *Conocarpus Erectus* Leaves on Nymphs and Adults of the Two-Spotted Spider Mite *Tetranychus urticae*

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Abstract. The research was carried out at the Beneficial Insects Laboratory in the Department of Biocontrol Technologies, College of Technical Engineering Al-Musayyib, Al-Furat Al-Awsat Technical University, which tested different concentration of *Conocarpus erectus* alcoholic extract (0.5, 1, 2, 3)% on the percentage of mortality of nymphs and adults of the two-spotted spider mite *Tetranychus urticae*. Shows the effective acaricidal property of the alcoholic extract which had caused 100% mortality of nymphs and adults at 3% concentration after seven days, while only 20% nymph mortality and 0% adult mortality was observed at 0.5% concentration. From the results presented, it is clear that the nymphs have more mortality compared to adults and also mortality rate increases with increasing concentration and time, it contains 10 bio active compounds according to GC-MS analysis.

Keywords: *Conocarpus erectus*; botanical acaricide; acaricidal activity; *T. Urticae* ; biopesticide.

1. INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: *Tetranychidae*), is one of the world's most damaging economic pests of crops. Highly polyphagous, this mite has been recorded feeding on more than 1500 species of plants and is known to attack many of the most important field and greenhouse crops including tomatoes, cucumbers, strawberries, soybeans and ornamentals [1], [2]. This cosmopolitan species can be found in many different climates and has been shown to produce extensive crop loss in a variety of systems in both temperate and tropical regions [3]. Damage by this mite is caused by the insertion of its stylet through plant cells in which it injects plant nutrients to then consume the resulting cell contents; damage caused by piercing and sucking results in characteristic chlorosis, bronzing and early defoliation which can lead to severe losses in photosynthetic capacity and crop yield [4]. Infestations are expected to increase under climate change scenarios; population growth of *T. Urticae* is highly

dependent on increasing temperatures due to accelerated reproductive and developmental rate with warmer conditions [5].

Perhaps the most important biological trait of *T. Urticae* contributing to its status as a virtually impossible to control pest, is the occurrence of arrhenotokous parthenogenesis, by which unfertilised females are capable of laying functional male eggs and a population of mites can therefore be founded from an individual [6]. This trait, coupled with very short development times, a very high rate of fecundity, and the ability to produce a number of generations within a season allow the production of rapid exponential population growth when environmental conditions are suitable [7]. Coupled with these biological traits they have rapid evolution rates to pesticide resistance. *T. Urticae* has shown the ability to evolve resistance to over 100 acaricide active ingredients and has now been declared "the most resistant arthropod in the world" [8],[9]. Target-site insensitivity, detoxification through metabolic systems mediated by cytochrome P450 monooxygenases, esterases and glutathione S-transferases, and diminished cuticular penetration rates have all been cited as the mechanism of resistance [10].

Chemical control is the most widely used method for the control of *T. Urticae* outbreaks. However, chemical control faces limitations in the last decades; overuse of artificial acaricides led to rapid selection for resistance and as result to the widespread failure of control measures which is reported in Europe, Asia, Middle East and America [9],[11]. Apart from resistance issues, chemical acaricides are serious ecological hazards. Indeed, they can lead to chemical residues in soil, water and non-target species, affect beneficial arthropods like predators, and pollinators, and accumulate in crops causing accumulation of toxic residues. Strict regulations exist in most countries and WHO demands for a more rapid use of ecological alternative pest control methods [13] since less chemical methods are available for resistance pests [11].

Plant extracts have become alternative or supplementary agents to synthetic acaricides within the integrated pest management strategy due to the biodegradability and low toxicity to mammals with varied phytochemical components that may retard resistance [14], [15]. Different studies demonstrated acaricidal efficacy of the plant extracts against *T. Urticae* [16], [17]. Among these plants, *Conocarpus erectus*, which belongs to a mangroves evergreen species, spread throughout tropical and subtropical areas in the world including Iraq. It contains active compounds including phenolics, flavonoids, tannins, alkaloids, gallic acid, quercetin and terpenoides [18], [19], exhibited activity against stored-products and sap sucking insects [20], [21]. Therefore, this experiment is designed to investigate acaricidal efficacy of various concentrations of alcoholic leaf extract of *Conocarpus erectus* against nymphs and adults of *T. Urticae* as environmental friendly botanical acaricide.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. PLANT MATERIAL

Fresh mature leaves of *Conocarpus erectus* were obtained from trees grown in Babylon Governorate, Iraq, in spring 2025. Leaves were washed with distilled water; then air-dried in cool shady place for two weeks until they were completely dried. Dried leaves were finely

powdered by electric mill into homogenously fine powder. Powder was kept in sealed glass containers at room temperature before extracting.

2.1.2. PREPARATION OF THE ALCOHOLIC EXTRACT

The extract was prepared using the Soxhlet apparatus in Beneficial Insects Laboratory of the Department of biocontrol technologies of the College of Technical engineering Al-Musayyib, Al-Furat Al-Awsat Technical University. 20 g of the dried leaf powder was introduced into cellulose thimble in the Soxhlet extractor and 200 mL of 99.97% absolute ethanol was used as solvent. The extraction was carried out continuously for 24 hours at 45°C. The obtained crude extract was then concentrated through removal of solvent by the rotary vacuum evaporator and then put into the weighed glass vials and then transferred into a thermal oven at 45°C until constant weight obtained to ensure complete removal of solvent. The dried extract was preserved in sealed glass vials at 5°C until used.

The stock solution of 10% (w/v) was prepared by dissolving 10 g of the dried extract in 5 ml of absolute ethyl alcohol and the volume was completed up to 100 ml by using distilled water with Tween 20 (1 mL in 100 mL) added to increase emulsion power. Different concentrations 0.5, 1, 2, and 3% (w/v) working solution was prepared from stock solution using serial dilution:

$$N_1V_1 = N_2V_2 \quad (1)$$

Where N_1 = concentration of the stock solution, V_1 = volume of stock solution required, N_2 = desired working concentration, and V_2 = final volume of the working solution.

2.1.3. MITE COLONY

A laboratory colony of *T.urticae* was obtained from adult mites collected from heavily infested plants from the experimental fields of Al-Musayyib Technical Institute / Al-Furat Al-Awsat Technical University. Mites were maintained by successive generations feeding on freshly excised castor bean (*Ricinus communis*) leaves. Castor bean leaves were floated adaxial side up on a wet layer of cotton wool in 9 cm Petri dishes, in order to keep the leaves turgid and serve as a water barrier; vaseline was placed on the margin of the leaf to keep the mites inside the Petri dishes, and to ensure ventilation the lid was punctured by a heated needle. Mite colony was maintained at controlled laboratory conditions of 26 °C, 60 5% R. H and light : dark period of 16:8 h.

3. METHODS

2.2.1. FIELD SURVEY

Field survey was conducted in Babylon governorate in 2025-2026 season, in four regions: District Al-Musayyib and sub districts Al-Kifl, Al-Jibla, and Abu Ghrai. Field visits was done weekly starting 30/4/2025 to 21/5/2025 and levels of infestation was measured at every visit. Each time 10 plants at the location have randomly selected, and inspect them, the percentage of infestation were taken.

2.2.2. BIOASSAY PROCEDURE

A laboratory bioassay was conducted in the laboratory to study the acaricidal activity of the alcoholic extract of *C. Erectus* against nymphs and adults of *T. Urticae* . 5 nymph or adult

individuals were transferred into each Petri dish with a newly supplied castor bean leaf disk. Various treatment concentrations (0.5, 1, 2 and 3 %) with a dose of 0.5ml per dish was sprayed on the dish using hand-operate atomizer sprayer. Distilled water plus Tween 20 was applied on the control group instead of any extract. All treatments were repeated three times, and then individuals responded with not moving to prick softly by the fine brush was counted as death. Records of results were taken at the time course of 1, 3, 5, and 7 days after treatment.

2.2.3. GC-MS ANALYSIS

GC-MS analysis was performed to find the chemical constituents of *C. Erectus* alcoholic extract. The GC-MS instrument used was Shimadzu QP2010 Plus; it was equipped with a capillary DB-5 column (30 m 0.25 mm 0.25 m) as the column, with helium as carrier gas at flow rate of 1.0 ml/min and the oven was operated at the range of temperature from 60°C to 280°C at the rate of 5°C/min. Mass spectra were compared with mass spectral libraries NIST/WILEY and only compounds with similarity score 75% were considered.

2.2.4. STATISTICAL ANALYSIS

The data were analyzed by two-way analysis of variance using the Statistical Analysis System (SAS, Version 9.4) [29]. The factors were: 1. Concentration of extracts (0.5, 1, 2 and 3% and control), 2. Day of observation (days 1, 3, 5 and 7). Least Significant Difference (LSD) test was applied to determine the differences between means at the 5% level of probability ($P < 0.05$). Percentage of mortality were corrected to remove natural mortality by Abbott's formula [28]:

$$\text{Corrected mortality (\%)} = [(T - C) / (100 - C)] \times 100 = \quad (2)$$

Where T=percent mortality in the treatment and C=percent mortality in the control. All experiments were performed under controlled laboratory conditions and were replicated twice to ensure consistency. Results are expressed as mean standard error of 3 replicates.

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. FIELD SURVEY RESULTS

Table 1 presents the results of the field survey for *T. Urticae* across four regions in Babylon Governorate during the 2025–2026 season.

Table 1. Field survey of the two-spotted spider mite *T. Urticae* in Babylon Governorate (2025–2026).

District / Sub-district	Visit Dates				District Mean (%)
	30/4	7/5	14/5	21/5	
Al-Musayyib District	40	30	20	15	26.25
Al-Kifl Sub-district	40	55	60	62	54.25
Al-Jibla Sub-district	20	30	45	40	33.75
Abu Ghraiq Sub-district	20	30	35	21	26.5
Overall Mean (%)	30	36.25	40	34.5	–

A range of infestation levels was observed during survey, in sampling site and dates. Al-Jibla Sub-district recorded highest percentage (60%, 62%) infestation rate on the 14th and 21st of May, where it was recorded 15% infestation rate on Al-Musayyib District on 21st of May (lowest recorded rate). The infestation mean values in the 4 survey dates were 30, 36.25, 40 and 34.5% respectively, and it had maximum activity rate in mid of May. All this can explain the preference behavior of the mite to the heat and dryness which is typical weather in Babylon in late spring. This is accordance with that stated that there is a high positive correlation between population development of *T. Urticae* and high temperature, (1, 4).

3.1.2. EFFECT OF THE ALCOHOLIC EXTRACT ON NYMPH MORTALITY

Table 2 shows the effect of the alcoholic extract of *Conocarpus erectus* leaves on the percentage mortality of *T. Urticae* nymphs across concentrations and observation days.

Table 2. Effect of the alcoholic leaf extract of *Conocarpus erectus* on nymph mortality (%) of *T. Urticae* .

Day	Concentration (%)	Replicates			Mean (%)
		A	B	C	
Day 1	0.5	0	0	0	0
	1	0	0	0	0
	2	20	0	0	6.7
	3	20	20	0	13.3
	Control	0	0	0	0
Day 3	0.5	0	0	0	0
	1	0	20	20	13.3
	2	20	20	20	20
	3	80	80	20	60
	Control	0	0	0	0
Day 5	0.5	0	20	20	13.3
	1	20	20	20	20
	2	80	20	80	60
	3	100	100	100	100
	Control	0	0	0	0
Day 7	0.5	20	20	20	20
	1	80	80	80	80
	2	80	80	80	80
	3	100	100	100	100
	Control	0	0	0	0

LSD: Days = 8.94 Replicates = 9.99 Interactions = 19.98

The results in Table 2 demonstrate the dose-response and time-response effects of the alcoholic extract of *C. Erectus* to *T. Urticae* nymphs. Maximum mortality of nymphs (100%) was observed at concentration of 3% at the 7th day; minimum mortality was 20% at 0.5% at the 7th day. At day 1 the activity of extract was not high but was significantly increases at the subsequent days, the reason may be attributed that the effective compounds need contact period to cause lethal effect.

3.1.3. EFFECT OF THE ALCOHOLIC EXTRACT ON ADULT MORTALITY

Table 3 presents the effect of the alcoholic extract of *Conocarpus erectus* leaves on the percentage mortality of *T. Urticae* adults.

Table 3. Effect of the alcoholic leaf extract of *Conocarpus erectus* on adult mortality (%) of *T. Urticae* .

Day	Concentration (%)	Replicates			Mean (%)
		A	B	C	
Day 1	0.5	0	0	0	0
	1	0	20	0	6.7
	2	20	20	0	13.3
	3	40	40	20	33.3
	Control	0	0	0	0
Day 3	0.5	0	0	0	0
	1	20	20	0	13.3
	2	40	60	40	46.7
	3	60	60	60	60
	Control	0	0	0	0
Day 5	0.5	20	0	0	6.7
	1	40	60	40	46.7
	2	60	40	40	46.7
	3	80	80	80	80
	Control	0	0	0	0
Day 7	0.5	0	0	0	0
	1	60	80	20	53.3
	2	80	100	60	80
	3	100	100	100	100
	Control	0	0	0	0

LSD: Days = 8.08 Replicates = 9.04 Interactions = 18.08

Table 3 also shows that adult mortality followed concentration- and time-dependent pattern. The maximal adult mortality of 100% occurred at the concentration of 3% after 7 days whereas the minimum was 0% at 0.5% after 7 days. Mortality of nymph was more pronounced than adult for corresponding concentrations and periods of time. This finding might have been correlated with mature cuticle and higher activity of detoxifying enzyme system of adults [22].

3.1.4. GC-MS ANALYSIS OF THE EXTRACT

GC-MS analysis of the alcoholic extract of *C. Erectus* leaves identified 10 bioactive compounds as presented in Table 4.

Table 4. GC-MS analysis results of the alcoholic leaf extract of *Conocarpus erectus*.

Peak	Compound Name	R-Time (min)	Area	Similarity (%)
1	Tetradecane	12.595	9,213,807	96
2	Tetradecane	12.674	8,712,796	96
3	Tetradecane	12.724	6,685,463	95

4	5-Hydroxymethylfurfural	13.208	91,165,713	95
5	5-Hydroxymethylfurfural	13.275	28,168,252	76
6	Hexadecane	14.842	21,018,881	97
7	Heptadecane	15.731	5,822,982	95
8	Hexadecane	16.557	9,553,993	88
9	Neophytadiene	17.001	4,845,553	94
10	Hexadecanoic acid, methyl ester	18.116	17,264,637	94

Ten compounds were identified by the GC-MS analysis; hexadecanoic acid methyl ester (methyl palmitate), neophytadiene and hexadecane being the major peaks. The presence of long-chain alkanes (tetradecane, hexadecane and heptadecane) and fatty acid methyl esters suggests a lipophilic bioactive fraction suitable for arthropod cuticle penetration.

3.2. DISCUSSION

The data obtained from the present study unambiguously showed that alcoholic extract of leaves of *C. Erectus* demonstrated promising acaricidal effect on nymph and adult stages of *T. Urticae*, with LC 100% at 3% and seven days post treatment. This is in accordance with the widely acknowledged dose-dependent action of botanical pesticides against mites. Similarly, Alpkent et al. [11] recorded concentration-dependent proportional mortality of *T. Urticae* using aqueous extract of different plant sources; confirming the concentration-dependent efficacy based on the type and concentration of bioactive ingredients. The greater susceptibility of nymph over adult observed in this experiment is also in agreement with the study of Stumpf et al. [22], who mentioned underdeveloped cuticle and limited metabolic detoxification system of young arthropods in relation to adults, as major causes of nymph resistance. The concentration-dependent mortality of day 1 through day 7 demonstrated here was suggestive of systemic cumulative toxicity instead of rapid knockdown which often complement many pesticidal botanicals rich in phenolics and terpenoids [23].

The GC-MS profile analysis revealed the presence of many phytochemical constituents in alcoholic extract of *C. Erectus*, the most significant compound based on retention time were hexadecanoic acid methyl ester followed by neophytadiene and long-chain alkanes. The hexadecanoic acid methyl ester (methyl palmitate) has been proven to be an effective acaricide for tetranychid mites and causes neuro-toxicity at the cellular level in the mite [24]. It is also one of the bioactive compounds responsible for pesticides activity in other botanical sources [25]. Neophytadiene, a diterpenoid that is the main constituent of the chlorophyll degradation pathway in the tissues of green plants has shown an insecticidal and repellent activity, through the impairment of nerve and enzymatic activity in target Arthropods [26]. The long chain alkanes like tetradecane, hexadecane, and heptadecane, were also identified, thus strengthening the assumption of cuticular penetration because these compounds are highly lipophilic, which can dissolve the lipid layers of insect cuticle [27].

The acaricidal activity demonstrated in this experiment against *T. Urticae* using the alcoholic extract of *C. Erectus* is also strongly supported by the broad phytochemical profile and medicinal properties of *Conocarpus* plant species. The presence of phenolic compound, antioxidant, gallic acid, ellagic acid, quercetin, myricetin etc, have been confirmed in *Conocarpus* species by Afifi et al. [18] which affects various enzymatic activities within

arthropod system. Several studies have also shown potent antioxidants, anticancer and antimicrobial effects in different parts of *Conocarpus* plants extracts which are majorly linked to the presence of phenols in these plant species [19]. Significant insecticidal activity on whitefly (*Bemisia tabaci*) and aphid (*Myzus persicae*) on eggplant has been reported in leaf extracts of *Conocarpus erectus* both in laboratory and field conditions [20]. Toxicity of *C. erectus* leaf extracts (both water and ethanol extract) against stored-product pest *T. castaneum* and its activity attributed to its alkaloid, phenol, and tannin components has been confirmed by Khashaveh et al. [21]. Therefore, *Conocarpus* species have versatile phytochemical compounds which act through a complementary mode of action.

Our observations are consistent with reports of botanical acaricides in previous works. Xie et al. [16] have demonstrated excellent acaricidal activity of *Atropa belladonna* on *T. Urticae*, in which a dose-related increase in the percentage of mortality was obtained which agrees with our results. Nasr Azadani et al. [17] have demonstrated that essential oil of *Artemisia annua* and its monoterpenes cause inhibition of detoxifying enzymes in *T. Urticae* to reduce tolerance of mites toward chemical pesticide, Benelli and Maggi [23] determined that phytochemical (secondary metabolites) of plants i.e. Phenols, alkaloid and terpenoids affect a variety of physiological pathways of arthropods and make resistance less likely to evolve [8]. Accordingly, it would be useful to propose that the alcohol extract of *C. erectus* could be a viable candidate in IPM programs to control *T. Urticae*. Further studies could address field application, the determination of LC values and the effect on natural enemies (e.g. *Phytoseiulus persimilis*). [15].

4. CONCLUSIONS

The ethanolic extract of *Conocarpus erectus* leaves showed dose and time dependent acaricidal activities at nymph and adult stages of *Tetranychus urticae*. It caused mortality up to 100% at concentration 3% in seven days in both stages where nymph was found to be more susceptible than adult in both stages. It contains ten compounds that include long chain alkanes, hexadecanoic acid methyl ester and neophytadiene, responsible for acaricidal activity as confirmed by GC-MS. The extract of this plant is a good alternative for chemical acaricides, should be explored to be used in the integrated pest management (IPM) program against *T. Urticae*.

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DECLARATION OF COMPETING INTEREST

None

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