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EFFECT OF *WARIONIA SAHARAE* EXTRACTS ON THE TWO-SPOTTED MITE *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE)

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ABSTRACT

This study evaluated the acaricidal and repellent effects of *Warionia saharae* extract on the two-spotted spider mite, *Tetranychus urticae*. Using five ethanolic concentrations (50%, 25%, 12.5%, 6%, and 1% (v/v), designated as C1, C2, C3, C4, and C5, respectively), the extract's efficacy was tested on adult mites. The results showed a significant increase in mortality over time and with higher concentration. At 72 h, all concentrations achieved 86-100% mortality. The highest concentrations (C1 and C2) were particularly fast-acting, causing over 69% mortality within 24 h. The extract also exhibited a repellent effect at the higher concentrations. The calculated LC50 values were 37.99% at 48 hours and 41.89% at 72 h. These findings suggest that *W. saharae* extract is a potent natural alternative for *controlling T. urticae*.

Keywords: *T. urticae*; *W. saharae*; acaricidal effect; repellency.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a highly polyphagous pest and is considered one of the most significant agricultural mites worldwide (Vignesh et al., 2019). This pest can infest a wide range of host plants, affecting more than 100 plant families and causing substantial yield losses (Idris et al., 2020; Leviticus et al., 2020; Van Leeuwen et al., 2015). *T. urticae* primarily feeds on leaf tissues, with feeding activity concentrated on the underside of leaves rather than the upper surface. This behavior is particularly important, as it often results in more severe damage on the lower leaf surface (Park & Lee, 2002). To control *T. urticae* populations, chemical acaricides are commonly applied. Despite their widespread use and significant effectiveness, these pesticides pose risks to human health, pollute ecosystems, promote the development of resistant mite populations, and may inadvertently eliminate beneficial arthropods, including predatory mites, disrupting ecological balance (Efrom et al., 2012). Therefore, developing natural and safer alternatives has become a priority in integrated pest management (Castillo-Ramírez et al., 2020; Gigon et al., 2016). Plant extracts have emerged as promising tools for managing various arthropods due to their well-documented biological properties. These natural compounds can induce mortality or alter pest behavior, contributing significantly to integrated pest management programs (de Araújo et al., 2020; Hikal et al., 2017; Nilahyane et al., 2012; Tembo et al., 2018; Qessaoui et al., 2024). Previous studies report that extracts and essential oils from plants are increasingly used as insecticides, particularly favored by organic growers and environmentally conscious consumers. These products possess repellent, insecticidal, antifeedant, growth-inhibiting, oviposition-deterring, ovicidal, and development-reducing effects on a wide range of insect pests (Basaid et al., 2020; Don-Pedro, 1996; Hikal et al., 2017; Odewole et al., 2020; Regnault-Roger et al., 2012).The objective of this study was to evaluate the toxicity and repellency effects of *Warionia saharae* extract against the adults of two-spotted mites *T. urticae.*

MATERIAL AND METHODS

Ethanol extracts of *W. saharae* The plants were collected from the Taznakht region in southeastern Morocco and transported to the INRA laboratory in Agadir. The leaves were air-dried in the dark for 7 days, then oven-dried at 40 °C for 3 days before being ground into a powder (Ait Taadaouit et al., 2012). An ethanolic extract was prepared by macerating 20 g of this powder in 200 mL of absolute ethanol with continuous shaking for 24 h at a laboratory temperature of 26±2 °C. The mixture was then filtered, and the solvent was evaporated using a rotary evaporator at 40°C. The resulting residue was stored at -20°C until needed (Ait Taadaouit et al., 2012). The yield was calculated using the following equation

$$\mathbf{R = \frac{M1}{M2} \times 100}$$

R: Yield (%), M1: Mass of the extract (g), and M2: Initial plant biomass (g)

Acaricidal activity

A laboratory bioassay was conducted to evaluate the acaricidal activity of *W. saharae* extract on adult *T. urticae* mites. Five concentrations of the ethanolic extract (50%, 25%, 12.5%, 6%, and 1%) were prepared. Homogeneous-age adult mites were sourced from the SAOAS company. The study employed a leaf-dip bioassay method (Bouharroud et al., 2006, 2007; Qessaoui et al., 2017, 2019, 2024), in which fresh tomato leaflets were dipped in either the test concentration or the control solution (sterile distilled water). Each treated leaflet was placed in a Petri dish leaf cage, and 15 adult *T. urticae* were transferred onto each leaflet. The experiment followed a randomized complete block design with four replicates per concentration across three runs. Mite mortality was recorded at 24, 48, and 72 h, and results were corrected for natural mortality using Abbott's formula (Abbott, 1925):

$$\mathbf{CrrM\%} = \frac{\mathbf{DMN - DMNC}}{\mathbf{MTN - DMNC}} \times \mathbf{100}$$

CrrM = corrected mortality, DMN = dead mite number, DMNC = Number of dead mites in control, and MTN = total mite number.

Repellency Test

A repellency test was conducted to evaluate the repellent effects of *W. saharae* extracts on adult *T. urticae* mites. The study used a two-chamber device connected by a hose, with one chamber containing treated leaves and the other containing control leaves (Qessaoui et al., 2017). Fifteen mites were introduced into the hose, allowing them to choose between the two chambers. Mite migration was recorded at 24, 48, and 72 h. The experiment was conducted as a randomized complete block design with four replicates , under controlled conditions of 25±1 °C, 55% relative humidity, and a 16:8 (L:D) photoperiod (Pascual-Villalobos & Robledo, 1998; Qessaoui et al., 2017, 2024). A repellency index was calculated using the formula of Pascual-Villalobos & Robledo (1998)

$$\mathbf{RI} = \frac{\mathbf{C - T}}{\mathbf{C + T}} \times \mathbf{100}$$

RI = repellency index, C = number *T. urticae* of adults in the control box, T = number *T. urticae* adults in the treated box

Statistical analysis

The efficacy of *W. saharae* extract on *T. urticae* mortality rates and repellent activities were subjected to one-way analysis of variance test (ANOVA), with Newman-Keuls test at $\alpha = 0.05$ using SPSS software. The probit parameters of the concentration-mortality responses were performed using the POLO-PC statistical software (Leora Software, 1987). The LC50's and their corresponding 95% confidence limit were calculated.

RESULT AND DISCUSSION

Yield of *W. saharae* extract obtained

The maceration extraction of *W. saharae* using 200 mL of ethanol as the solvent for 24 h yielded an average extract recovery of 15.36 ± 3.91%.

Acaricidal activity

All five concentrations of *W. saharae* extract caused highly significant mortality rates in adult *T. urticae* at 48 and 72 h after treatment (P = 0.001) (Fig. 1). Application of the *W. saharae* extract reduced mite mobility and induced a brownblack coloration in treated individuals. The results showed a linear relationship between mortality rate and both the concentration of *W. saharae* extract and the exposure time. The most rapid mortality (24 h after treatment) was observed at concentrations C1 and C2, with mortality rates of approximately 69%, while C3, C4, and C5 produced mortality levels of about 55%, 47%, and 31%, respectively.

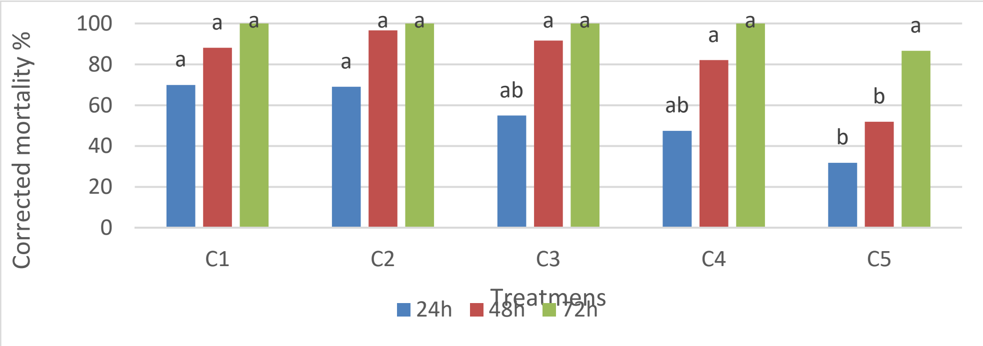

Figure 1: Effects of *W. saharae* extracts on adult T. urticae mortality rates; Bars with the same letters are not significantly different at $P < 0.05$ according to the Newman-Keuls test

The highest mortality rate (100%) after being treated with C1, C2, C3 and C4 was observed in due duration notably 72h. This was further confirmed by probit analysis indicating that the *W. saharae* extract provided the highest mortality rates, which had an LD_{50} of 30.95% 72h after treatment.

Repellency Test

The repellency effect of *W. saharae* extract was significant (P=0.001) against T. urticae adults for C1, C2, C3 and C4 at 24 and 48 after treatment (Fig.2).

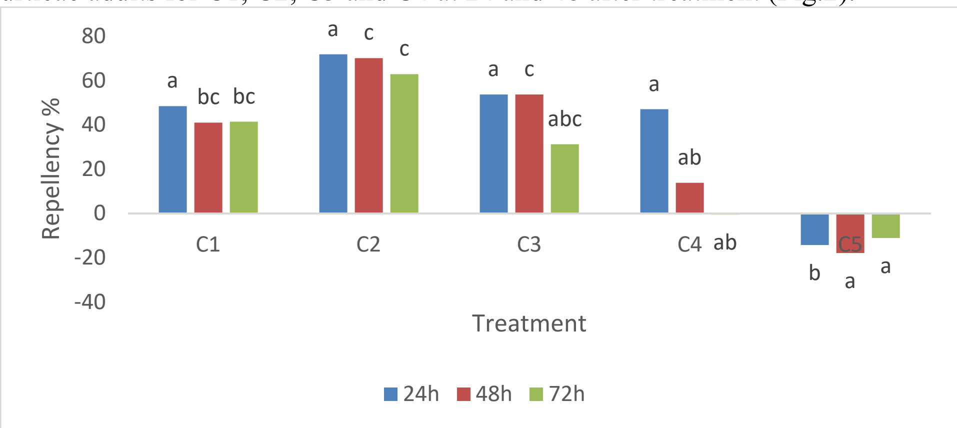

Figure 2: Percent repellency of *W. saharae* extract against T. urticae. Bars with the same letters are not significantly different at $P < 0.05$ according to the Newman-Keuls test

The repellence index (RI) produced by the three effective concentrations ranged from 47% to 71% for C4 and C1, respectively. In contrast, the lowest concentration, C5, did not exhibit any repellence effect (Fig. 2). Concentration C2 showed a consistently high repellence index throughout the entire exposure period, reaching 71%, 70%, and 62% at 24, 48, and 72 h, respectively. Overall, the repellence index of the tested concentrations was negatively correlated with the exposure period.
The results of this study indicate that *W. saharae* extract has a high efficacy against the phytophagous mite *T. urticae*. It possesses a significant acaricide ($P<0.01$) and repellent activity against the *T. urticae* adults. Our results show that all five concentrations (50%, 25%, 12,5%, 6% and 1%) of *W. saharae* extract provide significant rate of mortality in *T. urticae* adults. The higher mortality (100%) of *T. urticae* adults was observed 72h after treatments with C1 to C4 of *W. saharae* extract. The repellence index ranged from 13 to 70% for C4 and C1 respectively. Raja et al., (2001) reported that aqueous extracts from *C. rotundus* tubers effectively protected pulses against *Callosobruchus maculatus* (Coleoptera: Bruchidae). Barbosa et al., (2011) reported that alcohol extracts from *C. rotundus* show 55% of mortality and 28% leaf consumption of *Diabrotica speciosa* adults. The effect of *W. saharae* extract may be explained by the metabolites composition of this plant including volatile compounds. Many studies reported that *W. saharae* extract is rich in compounds such as polyphenols, alkaloids, anthraquinones, coumarins, steroids and triterpenes, sesquiterpenoid, flavonoids, saponins, tannins, glycosides, furochromones, monoterpenes, sitosterol, alkaloids saponins, terpenoids, essential oils, starch, carbohydrates, protein, separated amino acids, resins and many other secondary (Al-Snafi, 2016; Jeong et al., 2000; Kilani et al., 2005; Nagulendran et al., 2007). The extract of this plant has demonstrated insecticidal activity (Al-Snafi, 2016).

CONCLUSION

The present study demonstrated that ethanolic extracts of *W. saharae* possess strong acaricidal and repellent activities against the two-spotted spider mite, *T. urticae.* All tested concentrations induced significant mortality, with higher concentrations (C1 and C2) acting rapidly and achieving up to 100% mortality within 72 hours. In addition to its toxic effect, the extract exhibited a pronounced repellency, particularly at moderate to high concentrations, further enhancing its protective potential. The concentration- and time-dependent responses, supported by probit analysis and LC_{50} values, confirm the biological effectiveness of *W. saharae* extract. These bioactivities are likely related to the plant’s rich composition in secondary metabolites, including terpenoids, flavonoids, alkaloids, and other bioactive compounds known for their pesticidal properties. Overall, the findings highlight *W. saharae* as a promising natural alternative to synthetic acaricides. Its integration into integrated pest management (IPM) programs could contribute to reducing chemical pesticide use, limiting resistance development, and minimizing environmental and health risks. Further studies under greenhouse and field conditions, as well as investigations into formulation optimization and mode of action, are recommended to support its practical application.

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