Indian Journal of Entomology Online published Ref. No. e24939 DoI. No.: 10.55446/IJE.2024.939

POTENTIAL REPELLENCY AND TOXICITY OF HEXANE EXTRACTS FROM *CYPERUS ROTUNDUS* TO *TETRANYCHUS URTICAE* KOCH. (ACARI: TETRANYCHIDAE)

Redouan Qessaoui[1], Sokaina El Badaoui[1], Salahddine Chafiki[2], Abdelhadi Ajerrar[2], Badr Hajjaj[3], Rachid Elaini[4], Mohamed Alouani[5] and Rachid Bouharroud[1]*

[1]Regional Center of Agricultural Research of Agadir, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco
[2]AgroBioSciences Department (AgBS), Mohammed VI Polytechnic University (UM6P), Ben Guerir, Morocco
[3]Research and Development Unit, Regional Agricultural Research Center of Tangier, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco
[4]Macrobials Production Unit, Omnium Agricole du Souss, Industrial zone, Tassila 3, Agadir, Morocco
[5]Laboratory of Biotechnologies and Valorization of Natural Resources Faculty of Sciences - Agadir, Ibn Zohr University, B.P. 8106, 80000, Agadir, Morocco
*Email: rachid.bouharroud@inra.ma (corresponding author): ORCID ID 0000-0001-8914-1050

ABSTRACT

The objective of this work was to evaluate the acaricidal and repellency effect of *Cyperus rotundus* extract, an important weed plant, on *Tetranychus urticae* adults. Randomized complete block with 4 replicates was adopted. The hexane *C. rotundus* extract was obtained from tubers using the Soxhlet apparatus, then diluted to 5 concentrations (C1-100%, C2-50%, C3-25%, C4-10% and C5-1%), and a control (distillate water). Results showed that five concentrations caused significant mortality of *T. urticae* adults 24, 48 and 72 hr after treatment. The higher mortality was observed with C1 concentration of *C. rotundus* extract 72 hr after treatment (97%). Three concentrations C1, C2 and C3 showed a repellent effect on *T. urticae* adults. The repellence index ranged from 25 to 55% for C3 and C1, respectively. The present study demonstrated that the *C. rotundus* extracts could be an effective acaricide on *T. urticae* adults since the LC_{50} obtained was 30.95%.

Key words: Acaricide, biopesticides, integrated pest management, LC_{50}, *Tetranychus urticae*, plant extract, purple nutsedge, repellent, Soxhlet, toxic

Tetranychus urticae Koch (Acari: Tetranychidae), is a polyphagous pest and one of the most important agricultural mites around the world (Vignesh et al., 2019). Spider mites can infest plant species in more than 100 plant families causing significant yield losses (Van Leeuwen et al., 2015; Idris et al., 2020; Leviticus et al.,2020). *T. urticae* feed primarily on leaf surfaces, often occurring in higher numbers on the underside than on the upper side of leaves. This pest pierces the epidermis and feeds on the contents of mesophyll cells that resulting in chlorosis, causing a decrease in total chlorophyll content and an eventual loss of photosynthetic capacity (Park and Lee, 2002). To control *T. urticae*, the application of acaricides using adulticidal, ovicidal and nymphicidal, on the different life stages of two-spotted spider mites, is often used to reduce population levels. Moreover, acaricides can also eliminate many beneficial, including predatory mites causing biological imbalance (Efrom et al., 2012). They can also cause the development of pesticide-resistant mite populations. Biological control is a viable alternative to pesticides from both an environmental and economic perspective (Gigon et al., 2016; Castillo-Ramírez et al., 2020). The use of biological control agents, such as parasites, predators, bacteria or fungi can be used as alternative strategies for management (Ferrero et al., 2011; Gigon et al., 2016; Qessaoui et al., 2017, 2019, 2020; Castillo-Ramírez et al., 2020). Plant extracts are promising in the control of several arthropods due to their known biological properties, causing the death or affecting the behaviour of different agricultural pests (Nilahyane et al., 2012; Hikal et al., 2017; Tembo et al., 2018; de Araújo et al., 2020). Previous investigations report that the extract and essential oils extracted from aromatic plants have increased considerably as insecticides owing to their popularity with organic growers and environmentally conscious consumers. They have repellent, insecticidal, antifeedants, growth inhibitors, oviposition inhibitors, ovicides, and growth-reducing effects on a variety of insects (Don-Pedro 1996; Regnault-Roger et al., 2012; Hikal et al., 2017; Basaid et al., 2020; Odewole et al., 2020). Therefore, the objective of this study was to evaluate the efficacy of tuber extract of *C. rotundus* collected as a biopesticides against *T. urticae*.

MATERIALS AND METHODS

The tubers of the roots of *C. rotundus* were collected from a soil farm in Agdz, Zagora province, Drâa Tafilalet region, south of Morocco, and brought to the National Institute of Agronomic Research (INRA) laboratory in Agadir, Morocco. The fresh tubers were washed with water, dried in the shade, and then powdered. Twenty grams of *C. rotundus* powder was subjected to extract in a Soxhlet apparatus using hexane solvent (1: 3 (w/v)) (Singh et al., 2009). The extract was made solvent free using a rotary evaporator. The final residue was stored at −20°C (Kamala et al., 2018). The extract was dissolved in distilled water containing Tween 80 (0.05%). Five concentrations (C1(100%), C2(50%), C3(25%), C4(10%), and C5(1%)) were prepared. A control solution (C6) was also prepared using equal amounts of distilled water without the extract. Fresh tomato leaves were collected from plants growing in a greenhouse at the INRA experimental farm in Belfaa-Agadir, Morocco. The leaves were washed with sterile distilled water and dried under a laminar flow hood. To assess mortality effect, we evaluated the ability of five concentrations to cause mortality in homogeneous age adults of *T. urticae* on tomato leaves under laboratory conditions using a leaf-dip bioassay (Bouharroud et al., 2006, 2007; Qessaoui et al., 2017, 2019). Tomato leaves were immersed in each concentration for 20 s, dried under a laminar hood, and then transferred to leaf cages. The control leaves were dipped in control solution (C6). Each cage was populated with fifteen *T. urticae* adults, with (sex ratio 1:1.1, male: female). The cages were incubated at 24±2°C with a photoperiod of 16:8 (L:D). The mortality rate of *T. urticae* was evaluated at 24, 48, and 72 hr after treatment. The experiment was conducted using a randomized complete block design with four replications and three runs. The mortality rates were corrected using Abbott's formula (Abbott, 1925). To determine the repellent activity of *C. rotundus* extracts on *T. urticae* adults, a specific repellency test device was used (Qessaoui et al., 2017). Tomato leaves were immersed for 20 seconds in each concentration (C1 to C6), dried under a laminar flow hood, and then placed in the corresponding box in the repellent apparatus. Fifteen *T. urticae* adults were gently transferred into the hole pierced in the middle of the linked hose and then sealed (Pascual-Villalobos and Robledo 1998; Qessaoui et al., 2017). The experiment followed a randomized complete block design with four replications and three runs. The box was incubated at 24±2°C with a photoperiod of 16:8 (L:D) and 55% of relative humidity. A repellency index was calculated at 24, 48, and 72 hr after treatement using the formula of Pascual-Villalobos & Robledo (1998). The data were subjected to one-way ANOVA at $p \leq 0.05$ using statistica software (V 10). The resulting means were compared using the Newman-Keuls test (p=0.05).

RESULTS AND DISCUSSION

The results indicate that all five concentrations of *C. rotundus* extract provided significant amounts of mortality to *T. urticae* adults for 24, 48 and 72 hr after treatment (Table 1). The symptoms caused by *C. rotundus* extract on *T. urticae* were reducing movement and the occurrence of brown-black coloration (Aksoy et al., 2009). The result shows a linear relationship between mortality rate and concentrations of *C. rotundus* extract. The most rapid *T. urticae* deaths (24 hr after treatment) was obtained by concentration

Table 1. Effects of *C. rotundus* extracts on *T. urticae* adult mortality rates

Concentration %	Hours after application		
	24 hr*	48 hr	72 hr
C5 (1%)	08.30 ± 24.89^{a}	16.97 ± 22.47^{a}	37.30 ± 32.00^{a}
C4 (10%)	14.55 ± 37.12^{a}	43.83 ± 36.49^{b}	54.76 ± 27.13^{ab}
C3 (25%)	44.83 ± 37.22^{b}	63.33 ± 25.29^{bc}	66.02 ± 26.16^{b}
C2 (50%)	58.76 ± 27.18^{b}	70.24 ± 23.97^{bc}	74.13 ± 16.67^{b}
C1 (100%)	65.37 ± 39.22^{b}	78.50 ± 33.21^{c}	96.65 ± 06.55^{c}
P-value	0.00029	0.000078	0.000004

*By column, the rates followed by the same letters are not statistically different at P<5% according to the Newman-Keuls test. Data for adult mortality rates are presented as the means ± standard deviation

C1 which had a mortality rate of about 65%, while concentration C2, concentration C3, concentration C4 and concentration C5 had approximately 58, 44, 14 and 8% mortality rates, respectively. The highest mortality rate (96%) was observed 72 hr after treatment by C1. For the other concentrations (C2, C3, C4 and C5), the mortality rates, 72 hr after application, were more than 50% except C5. This was further confirmed by probit analysis indicating that the *C. rotundus* extract provided the highest mortality rates, which had an LD_{50} of 30.95% 72 hr after treatment (Table 2). The consolidated data of the repellency observed in different concentrations of *C. rotundus* extract is given and it is evident that among those five concentrations, C1, C2 and C3 have a repellent effect to *T. urticae* adults at 24, 48 and 72 hr. The repellence index (RI) of the three concentrations ranging from 25 to 55% for C3 and C1 respectively. While C4 and C5 have not provided any repellent effect (Fig. 1).

This result concurred with those report that the methanolic root extract of *C. rotundus* and its fractions (n-hexane, chloroform, n-butanol, and aqueous) showed significant insecticidal activity against nymphs of *Aphis craccivora* Koch and crawlers of *Planococcus lilacinus* (Cockerell) (Singh et al., 2023). Also, Elhaj et al. (2021) reported that *C. rotundus* with Sesame Oil showed a significant insecticidal activity against African bollworm *Helicoverpa armigera Hübner* (Lepidoptera: Noctuidae). Furthermore, El Moghazy and El-Namaky (2019) showed that *C. rotundus* Oils have a significant larvicidal effect on the third instar larvae *Cephalopina titillator* (L.). Moreover, Barbosa et al. (2011) reported that alcohol extracts from *C. rotundus* show 55% of mortality and 28% leaf consumption of *D. speciosa* adults. Singh et al. (2009) reported that the tuber extracts of *C. rotundus* are more effective for repellency of all the mosquito vector even at low doses, and repellency rates varied between 80 and 100% for different repellents concentrations (2.5, 5, and 10%). Janaki et al. (2018) report that essential oil of *C. rotundus* showed an insecticidal effect against adults *Callosobruchus maculatus* F., *Oryzaephilus surinamensis* L., and *Trogoderma granarium* Everts. In the same context, Bañez and Castor (2011) reported that essential oil of *C. rotundus* showed an insecticidal effect more effective than carbamate pesticides in terms of insecticidal properties against ants, aphids, flies, and cockroaches, and is similar to organophosphates in terms of efficiency. The insecticidal effect of *C. rotundus extract* may be explained by the metabolites composition of this plant including volatile metabolites which kept mites from treated leaves in our study. Many studies report that *C. rotundus* extract is rich on 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; Hassan and Wahba, 2023; Gomathi and Maneemegalai, 2023; and Xue et al., 2023). This extract act as potential biopesticides and may be applied in integrated pest management programs to reduce the amount of synthetic chemical products being used to control plant pests and diseases. Many horticultural crop growers

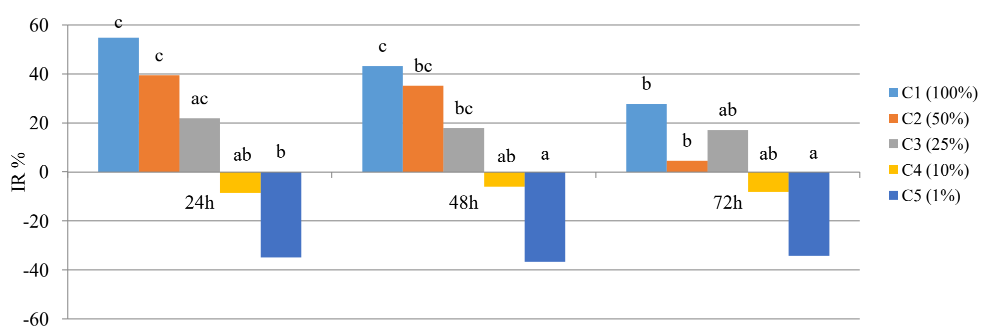

Fig. 1. Repellency of hexane extract of tuber of root of *C. rotundus* against *T. urticae* adults.
(Bars with the same letters not significantly different at $p<0.05$ according to the Newman-Keuls test)

Table 2. Log-dose probit mortality data for *T. urticae* adults tested with *C. rotundus* extract 72 hr after treatment

	LC_{50} (%)	Fiducial limits	Slope± SE	chi-square	Heterogeneity	DF
C. rotundus extract	30.95	18.64 - 40.44	2.849± 0.763	11.25	0.87	13

throughout the world are now exploring their potential applicability due to ongoing pesticide restrictions. Results from our study further suggest that *C. rotundus* extracts are of interest for use in the biological control of pests and could also be examined for their potential use in insect pest management.

ACKNOWLEDGEMENTS

We acknowledge Abderrahim Amarraque and Yassine Imlil for their technical assistance. We are grateful for the support provided by SAOAS.

AUTHOR CONTRIBUTION STATEMENT

RQ and RB: Conceptualization, Preparation, Methodology, Bioassays and Supervision. SEB, SC and AA: Preparation, Methodology and Bioassays. RE: Mites rearing. RQ, RE, BH, MA and RB: Writing-Reviewing and Editing.

FINANCIAL SUPPORT

No funding received.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Abbott W S. 1925. A method of computing effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Aksoy H M, Ozman-Sullivan S K, Ocal H, Celik N, Sullivan G T. 2009. The effects of *Pseudomonas putida* biotype B on *Tetranychus urticae* (*Acari: Tetranychidae*). Diseases of Mites and Ticks 46:223–230.

Bañez S E S, Castor L. 2011. Phytochemical and pesticidal properties of barsanga (*Cyperus rotundus Linn.*). JPAIR Multidisciplinary Research Journal 6(1): 1-1.

Barbosa F S, Leite G L D, Paulino M A D O, Guilherme D D O, Maia J T L S, Fernandes R C. 2011. Toxicity of extracts of *Cyperus rotundus* on *Diabrotica speciosa*. Acta Scientiarum. Agronomy 33: 607-611.

Basaid K, Chebli B, Mayad E H, Furze J N, Bouharroud R, Krier F, Barakate M, Paulitz T. 2020. Biological activities of essential oils and lipopeptides applied to control plant pests and diseases: a review. International Journal of Pest Management 67(2): 155-177.

Bouharroud R, Hanafi A, Brown J K, Serghini M A. 2006. Resistance and cross-resistance of *Bemisia tabaci* to three commonly used insecticides in the tomato greenhouses of the Souss Valley of Morocco. European Journal of Scientific Research 14: 587-594.

Bouharroud R, Hanafi A, Serghini M A. 2007. Pyrethroids and endosulfan resistance of *Bemisia tabaci* in the tomato greenhouses of the souss valley of morocco. Advances in Soil and Soilless Cultivation under 747: 409-413.

Castillo-Ramírez O, Guzmán-Franco A W, Santillán-Galicia M T, Tamayo-Mejía F. 2020. Interaction between predatory mites (*Acari: Phytoseiidae*) and entomopathogenic fungi in *Tetranychus urticae* populations. BioControl 65(4): 433-445.

de Araújo M J C, da Câmara C A G, Born F de S, de Moraes M M. 2020. Acaricidal activity of binary blends of essential oils and selected constituents against *Tetranychus urticae* in laboratory/ greenhouse experiments and the impact on *Neoseiulus californicus.* Experimental and Applied Acarology 80: 423-444.

Don-Pedro K N. 1996. Investigation of single and joint fumigant insecticidal action of citruspeel oil components. Journal of Pesticide Science 46: 79-84.

Efrom C F S, Redaelli L R, Meirelles R N, Ourique C B. 2012. Side-effects of pesticides used in the organic system of production on *Apis mellifera* linnaeus, 1758. Brazilian Archives of Biology and Technology 55: 47-53.

Elhaj W E, Osman A A, Elawad L M E. 2021. Insecticidal Activity of Cyperus rotundus L. and Datura stramonium L. Co-Administered with Sesame Oil against African Bollworm Helicoverpa armigera Hübner (Lepidoptera: Noctuidae). Journal of Agronomy Research 3(4): 1-8.

El Moghazy, M F, H El-Namaky A. 2019. Larvicidal effect of zingiber officinale and Cyperusrotundus oils on the third instar larvae *Cephalopina titillator* (L.). Egyptian Journal of Veterinary Sciences 29-37.

Ferrero M, Calvo F J, Atuahiva T, Tixier M S, Kreiter S. 2011. Biological control of *Tetranychus evansi* Baker and Pritchard and *Tetranychus urticae* Koch by *Phytoseiulus longipes* Evans in tomato greenhouses in Spain (Acari: Tetranychidae, Phytoseiidae). Biological Control 58: 30-35.

Gigon V, Camps C, Le Corff J. 2016. Biological control of *Tetranychus urticae* by *Phytoseiulus macropilis* and *Macrolophus pygmaeus* in tomato greenhouses. Experimental and Applied Acarology 68: 55-70.

Gomathi S, Maneemegalai S. 2023. Phytochemical analysis and in vitro anti-oxidant activities of medicinal plants *Cyperus rotundus*, *Tinospora cordifolia* and their formulation. Oriental Journal of Chemistry 39(3).

Hassan N A E, Wahba T F. 2023. Chemical profile, antifeedant, insecticidal activities, and some biochemical properties of two essential oils, Cyperus and Jojoba, against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Journal of the Advances in Agricultural Researches 28(2): 492-499.

Hikal W M, Baeshen R S, Said-Al Ahl H A H. 2017. Botanical insecticide as simple extractives for pest control. Cogent Biology 3: 1404274.

Idris A L, Fan X, Muhammad M H, Guo Y, Guan X, Huang T. 2020. Ecologically controlling insect and mite pests of tea plants with microbial pesticides: a review. Archives of Microbiology 202: 1275-1284.

Janaki S, Zandi-Sohani N, Ramezani L, Szumny A. 2018. Chemical composition and insecticidal efficacy of *Cyperus rotundus* essential oil against three stored product pests. International Biodeterioration and Biodegradation | Journal 133: 93-98.

Kamala A, Middha S K, Gopinath C, Sindhura H S, Karigar C. 2018. In vitro antioxidant potentials of *Cyperus rotundus* L. rhizome extracts and their phytochemical analysis. Pharmacognosy Magazine 14: 261-267.

Leviticus K, Cui L, Ling H, Jia Z, Huang Q, Han Z, Zhao C, Xu L. 2020. Lethal and sublethal effects of fluralaner on the two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae). Pest Management Science 76: 888-893.

Nilahyane A, Bouharroud R, Hormatallah A, Taadaouit N A. 2012. Larvicidal effect of plant extracts on *Tuta absoluta* (Lepidoptera, Gelechiidae). IOBC–WRPS Bulletin 80: 305-310.

Odewole A F, Adebayo T A, Babarinde S A, Awolokun G S. 2020.

Insecticidal activity of aqueous indigenous plant extracts against insect pests associated with cucumber (*Cucumis sativus* L.) in Southern Guinea Savannah Zone of Nigeria. Archives of Phytopathology and Plant Protection 53(5-6): 230-246.

Park Y L, Lee J H. 2002. Leaf cell and tissue damage of cucumber caused by two spotted spider mite (Acari: *Tetranychidae*). Journal of Economic Entomology 95: 952-957.

Pascual-Villalobos M J, Robledo A. 1998. Screening for anti-insect activity in Mediterranean plants. Industrial Crops and Products 8: 183-194.

Qessaoui R, Amarraque A, Lahmyed H, Ajerrar A, Mayad E H, Chebli B, Walters A S, Bouharroud R. 2020. Inoculation of tomato plants with rhizobacteria suppresses development of whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae): Agro-ecological application. PLoS One 15: e0231496.

Qessaoui R, Bouharroud R, Amarraque A, Ajerrar A, El Hassan M, Chebli B, Dadi M, Elaini R, El Filali F, Walters A S. 2017. Ecological applications of *Pseudomonas* as a biopesticide to control two-spotted mite *Tetranychus urticae*: Chitinase and HCN production. Journal of Plant Protection Research 57(4).

Qessaoui R, Rachid B, Abderahim A, Hind L, Abdelhadi A, Naima A A, Abdelghani T, El Hassan M, Bouchra C. 2019. Effect of *Pseudomonas* as a preventive and curative control of tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). Journal of Applied Sciences 19(5): 473-479.

Regnault-Roger C, Vincent C, Arnason J T. 2012. Essential oils in insect control: low-risk products in a high-stakes world. Annual Review of Entomology 57: 405-424.

Singh R, Gupta H, Aggarwal G, Bhattacharyya K, Sharma U, Reddy S E. 2023. Cyperus rotundus L.: Invasive weed plant with insecticidal potential against Aphis craccivora Koch and Planococcus lilacinus (Cockerell). Pesticide Biochemistry and Physiology 105720.

Singh S P, Raghavendra K, Dash A P. 2009. Evaluation of hexane extract of tuber of root of *Cyperus rotundus* Linn (Cyperaceae) for Repellency against mosquito vectors. Journal of Parasitology Research 2009: 1-5.

Tembo Y, Mkindi A G, Mkenda P A, Mpumi N, Mwanauta R, Stevenson P C, Ndakidemi P A, Belmain S R. 2018. Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. Frontiers in Plant Science 9: 1425.

Van Leeuwen T, Tirry L, Yamamoto A, Nauen R, Dermauw W. 2015. The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pesticide Biochemistry and Physiology 121: 12-21.

Vignesh M, Patil R K, Udhayakumar V S. 2019. Evaluation for promising biorationals and their synergetic combination against the two-spotted spider mite *Tetranychus urticae* Koch. Journal of Entomology and Zoology Studies 7: 356-360.

Xue B, He R S, Lai J X, Mireku-Gyimah N A, Zhang L H, Wu H H. 2023. Phytochemistry, data mining, pharmacology, toxicology and the analytical methods of *Cyperus rotundus* L. (Cyperaceae): a comprehensive review. Phytochemistry Reviews pp. 1-46.

(Manuscript Received: November, 2023; Revised: January, 2024;
Accepted: February, 2024; Online Published: March, 2024)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e24939