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Oviposition inhibitory activity of the Mexican sunflower *Tithonia diversifolia* (Asteraceae) polar extracts against the two-spotted spider mite *Tetranychus urticae* (Tetranychidae)

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ABSTRACT

The Mexican sunflower (*Tithonia diversifolia*, Asteraceae) is an invasive shrub of agricultural and non-agricultural lands in tropical countries. Besides extensive utilizations in the traditional medicine, mainly to treat malaria, the plant is believed to have a great potential in agriculture of developing countries as a green biomass to produce fertilizer, fodder and biopesticides. The plant is known to produce tagitinins, which are sesquiterpene lactones with a bitter taste endowed with toxicity against several insects such as mosquitoes, aphids, and beetles. Here, we evaluated the potential of *T. diversifolia* against the two-spotted spider mite *Tetranychus urticae* (Tetranychidae), which is one of the most economically important arthropod pests worldwide. The leaf methanolic extract and its ethyl acetate fraction were tested for acute and chronic toxicity and for oviposition inhibitory effects. The chemical composition of the extracts was analyzed by HPLC-MS^n and NMR. The main constituents were flavonoid derivatives, phenylpropanoids and sesquiterpene lactones. Among the latter, tagitinin C and tagitinin A were the major compounds. In acute toxicity assays, mortality did not exceed 50% even for the highest tested dose of 150 μg cm^{-3}. However, in chronic toxicity assays, on day 5 from application, the methanolic extract LD_{50} was 41.3 μg cm^{-3} while LD_{90} was 98.7 μg cm^{-3}. Furthermore, both *T. diversifolia* extracts inhibited oviposition in *T. urticae*. The ethyl acetate extract was the most active oviposition inhibitor, with an ED_{50} value of 44.3 μg cm^{-3} and an ED_{90} of 121.5 μg cm^{-3}. Overall, the good yield rate of the extract and the high crop yield highlighted good prospects of using the extract from this plant for the development of oviposition inhibitors against mites.

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Tetranychidae), is considered as one of the most economically important arthropod pests. This mite has been reported infesting over 1200 species of plants, including cereals, legumes (with special reference to soybean), greenhouse crops, ornamental plants and fruit trees [21,49,53]. The high number of population outbreaks registered for this species are mainly due to its rapid population growth, short developmental time and long adult survival [56]. This features, coupled with male haploidy, which exposes recessive resistance genes to selection, result in a high rate of development of resistance

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to acaricides. Indeed, in more than 60 countries, it has been noted that the mites have developed resistance to more than 80 acaricides [24,43]. Therefore, the reduction of the employ of synthetic acaricides in Integrated Pest Management programs is crucial, and it is particularly recommended to alternate them with products showing different mechanism(s) of action [18,30,38]. In this framework, plant-borne products recently emerged as potential novel control tools to be used against arthropod pests, including mites [10,14,44–46].

Tithonia diversifolia (Hemsl.) A. Gray, also known as Mexican sunflower, is a shrub native to Central America and currently naturalized in tropical regions of Africa, Asia and South America. The plant is considered as an invasive species in cultivated and non-cultivated lands [6,13]. In tropical regions, given its abundance and availability, *T. diversifolia* is widely used in the traditional medicine to treat several diseases [26]. Among them, the most common therapeutic use is to cure malaria after oral administration of its decoction [27,39]. This traditional use has been validated through scientific studies [3,20,25,42] where the bioactive constituents were identified as the sesquiterpene lactones, named tagitinins [22,25]. Other traditional therapeutic uses of the plant concern the treatment of microbial infections and snakebites in Africa [34,39], diabetes in Asia [36], and skin diseases in America [26].

Given its capability to grow up very quickly, *T. diversifolia* has been experimented in agriculture to improve soil fertility [31] and as fodder for broiler chickens [19]. Recently, the *T. diversifolia* extracts attracted attention of scientists as useful tools in crop protection. This perspective of use was borne from the observation that some herbivores such as the caterpillar *Chlosyne lacinia* avoid the plant secretory structures where the bitter sesquiterpene lactones are produced [4]. These preliminary results were substantiated by other studies. For instance, the methanolic extract of leaves has been tested against the generalist phytophagous *Atta cephalotes*, showing insecticidal effects by both ingestion and contact [12]. Mikenda et al. [35] found that *T. diversifolia* extract is active against key pest species (e.g. aphids and beetles) on common bean plants (*Phaseoulus vulgaris*). Adedire and Akinneye [2] tested the leaf ethanolic extract of *T. diversifolia* on the cowpea seed beetle *Callosobruchus maculatus* that infests commonly stored legumes and found it highly effective on oviposition, adult emergence and mortality. Radhakrishnan and Prabhakaran [48] showed that the aqueous extract of the plant possesses moderate effect on the red spider mite *Oligonychus coffeae*, which is one of the major pests infesting tea plantations.

Nowadays, there is an urgent need to develop newer and safer control tools against arthropods of agricultural and medical importance [7–9,29,47]. In this scenario, continuing our investigations on the potential application of *T. diversifolia* on the industrial level [41], here we focused on the evaluation of the acaricidal and oviposition inhibitory activity of the *T. diversifolia* polar extracts against the two-spotted spider mite *T. urticae* Koch (Tetranychidae), relying to acute and chronic toxicity tests, as well as to experiments evaluating the oviposition inhibition potential. The chemical composition of the extracts was achieved by NMR and HPLC-MS measurements (i.e. HSQC-DEPT, HPLC-ESI-MS and HPLC-DAD) and correlated with the biological activity.

2. Material and methods

2.1. Plant material

Leaves of *T. diversifolia* were gathered in Dschang, western region of Cameroon (N 05°26'18″, E 10°04'07″, 1450 m a.s.l), by one of us (P.C. Biapa Nya) in January 2016 during the dry season. Botanical authentication was performed by taxonomist Mr. Nana and a voucher specimen has been stored at the National Herbarium of Yaoundé, Cameroon, under the code 10196/HNC. Before extraction, leaves were air-dried in the dark at room temperature (≈25 °C) for 3 days and stored in wrapping papers.

2.2. Chemicals and reagents

Methanol and ethyl acetate were purchased from Sigma-Aldrich (Milan, Italy). HPLC grade acetonitrile was obtained from J. T. Baker (Phillipsburg, USA). HPLC-grade formic acid was purchased from Dikma Tech. Inc. (Beijing, China). Water (H_2O) was purified by a Milli-Q system (Millipore, Billerica, MA, USA) in our laboratory.

2.3. Preparation of extracts

Fifty grams of leaves were reduced into powder and macerated in 500 ml of methanol for 24 h. After filtration using cotton, the extract was concentrated under reduced pressure at 30 °C with a rotary evaporator and freeze-dried yielding 2.64 g crude extract (yield 5.28%). A portion of the methanolic extract (0.82 g) was macerated in 150 ml of ethyl acetate for 24 h, yielding, after filtration and evaporation, 0.38 g (46.3%) of a pasty ethyl acetate phase. The obtained extracts were stored in glass vials protected from light at −20 °C before chemical characterization and acaricidal experiments.

2.4. Experimental apparatus and chromatographic conditions

Analysis of *T. diversifolia* secondary metabolites was performed on a LC–MS system. LC–MS equipment (Varian) comprised a binary chromatographic system (Varian LC-212) coupled with a mass spectrometer Varian 500-MS (ion trap). An ion source electrospray (ESI) (Varian) was used. The MS parameters were as follows: needle potential −5.0 kV, shield 600 V, spray chamber temperature 50 °C, drying gas pressure 10 psi, drying gas temperature 350 °C, capillary voltage 80 V, RF loading 100, MS range 150–2000 Da. MS^n spectra were recorded during the chromatography run by using of the turbo-dds (tdds) utility giving the MS fragmentation pathways of ionic species whose intensity was higher than a threshold level. HPLC-DAD analysis was carried out by an Agilent 1100 series liquid chromatograph equipped with autosampler (Agilent 1100 series) and Diode Array Detector (DAD) (Agilent 1100 series). An Eclipse XDB-C8 5 μm 4.6 × 150 mm (Agilent) column was used as stationary phase. Mobile phases were: aqueous formic acid (0.1%) (A) and acetonitrile (B). The gradient elution was as follows: 0–30 min, linear gradient from 10% to 100% of B; 30–35 min, isocratic conditions at 100% of B; 35–36 min, linear gradient from 100% to 10% of B; 36–40 min, isocratic conditions at 10% of B. Flow rate: 1 ml/min. Calibration curves were obtained by standard solutions of rutin for flavonoid derivatives (at 350 nm) and chlorogenic acid for caffeoylquinic acid derivatives (at 330 nm). The concentration ranges were 11.7–117 μg/ml and 13.2–132 μg/ml for chlorogenic acid and rutin, respectively. The limits of detection (LOD) and quantification (LOQ) were 1.5 and 4.0 μg/ml, and 0.5 and 1.5 μg/ml for chlorogenic acid and rutin, respectively.

2.5. Qualitative and quantitative NMR analysis

NMR analysis was obtained on a Bruker AVANCE III spectrometer operating at 400.13 MHz for ^1H NMR and 100 MHz for ^{13}C. 2D spectra, HSQC-DEPT, HMBC, COSY and TOCSY were used for compound identification in mixture. Samples were dissolved in deuterated methanol and used for analysis. For quantitative purposes, previously published conditions were used [16]. Briefly, extract was dissolved in deuterated chloroform at a final

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