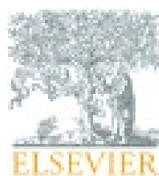

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Acaricidal activity of different extracts from *Syzygium cumini* L. Skeels (Pomposia) against *Tetranychus urticae* Koch

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ABSTRACT

Objective: To investigate the acaricidal activity of different extracts from *Syzygium cumini* (*S. cumini*) (Pomposia) againsst *Tetranychus urticae* Koch (*T. urticae*) and the biochemical changes in antioxidants enzymes. **Methods:** Six extracts of *S. cumini* (Pomposia) at concentrations of 75, 150 and 300 μ g/mL were used to control *T. urticae* (Koch). **Results:** The ethanol extract showed the most efficient acaricidal activity agent against *T. urticae* (98.5%) followed by hexane extract (94.0%), ether and ethyl acetate extract (90.0%). The LC_{50} values of the promising extract were 85.0, 101.0, 102.0 and 98.0 μ g/mL, respectively. The activities of enzymes including ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in susceptible mites were increased. The activities of all antioxidant enzymes reach the maximum value in mites at LC_{50} with ethanol and ethyl acetate extracts, respectively. **Conclusions:** The extract of *S. cumini* has acaricidal acivity against *T. urticae*, and the ethanol extract is the most efficient.

1. Introduction

Due to problems associated with the use of synthetic insecticides, researchers began to look for natural plant protection compounds such as botanical insecticides and antifeedants. Botanical products are useful tools in many pest management programs because they are effective and specifically target plants natural enemies[1]. Phytophagous mites, such as *Tetranychus urticae* (*T. urticae*) Koch, are one of the major pests in Egypt, attacking cotton, fruit trees and vegetables. It usually feeds on the leaves whose epidermis is damaged, resulting in yellow, brown blotch accompanied by dry leaf-fall. A severe mite-feeding results in reduction in both the quality and quantity of the crop. Control of *T. urticae* in Egypt has been almost exclusively focused on pesticides. Unfortunately, spider mites have developed resistance to most available pesticides and the loss of acaricidal efficacy as a result of resistance mite populations in the major problem encountered[2]. There is no doubt that widespread indiscriminate pesticide application causes pollution to the health and hinders the control process. So, the intensive use of acaricides in the last few years is not acceptable according to the modern criteria of integrated pest management (IPM) programs, leading to an increasing interest in alternative pesticides which derived from natural plants[3].

Several species of mite killers, including some predatory mites living on the host plants, normally keep these mite populations below damaging levels[4]. However, pesticides used to control other pests also kill these beneficial insects. This decimation of the natural enemy coupled with high reproductive potential and a short life cycle of the pest mites can lead to a rapid development of outbreaks. Acaricides used to minimize the impact are often more toxic to natural enemies of mites, and their application may actually aggravate the problem. Furthermore, control of spider mites has become increasingly difficult due to their resistance to many common synthetic pesticides[5]. Given the imposed quality restrictions on fresh market fruit, new pesticides that are effective against phytophagous mites and nontoxic to their natural enemies are urgently needed to combat these mites in the world. Acaricidal bioactivities of *Plumbago zeylanica* L. root extracts against *Panonychus citri* (*P. citri*) were excellent in adulticidal, ovicidal and oviposit inhibition[6]. The corrected mortality of *Eupatorium adenophorm* ethanol extracts (0.1% w/v) against *P. citri* was 71.10% and 73.53%, respectively, at 12 and 24 h

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after treatment[7]. Middle Eastern flora was used as a source of new safe bio-acaricides to control *T. cinnabarinus*[8]. Benzene and hexane extracts of *Syringa vulgaris* was also showed strong acaricidal bioactivities[9].

Survival of organisms and micro-organisms in aerobic environments has been associated with enzymatic and non-enzymatic mechanisms that help in avoiding or destroying toxic oxygen or reactive oxygen species (ROS). These oxygen forms have been reported to interact with vital macromolecules (inactivation of enzymes, damage to nucleic acids) and cell membrane components (lipid peroxidation) [10,11]. Endogenously- produced ROS, including hydrogen peroxide (H_2O_2), superoxide anion radicals (O_2^-), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$), may be intercepted by various cellular antioxidants such as reduced glutathione (GSH), ascorbate (AsA), β-carotene, α-tocopherol or uric acid[10]. Potential oxidative damage and stress can be alleviated by scavenging or changing the chemical identity of ROS. Partially reduced oxygen species (O_2^-, H_2O_2) may be endogenously generated by electron transport processes that are mediated through mitochondrial or microsomal enzymes, and via the photosynthetic pathways[12]. They can be also formed by certain organic xenobiotics, such as pesticides[13-15] or like the herbicide paraquat or by pro-oxidant plant allelochemicals such as the furanocoumarin xanthotoxin or the flavanoid quercetin[16]. Herbivorous organisms including insects and mites may obtain appreciable amounts of hydrogen peroxide molecules from plant materials[17].

By changing the identity and reactivity of toxic oxygen species, enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidases (POD) including glutathione peroxidase (GPOX) and ascorbate peroxidase (APX), and glutathione reductase (GR) make up another line of defence[18]. Imbalance between production and hazardous ROS and sufficient enzymatic and non-enzymatic protection has been implicated in senescence as well as in a variety of diseases and pathological disorders[11, 19].

Syzygium cumini (*S. cumini*) (L.) fruits, Skeels (Black Plum) are edible and are reported to contain vitamin C, gallic acid, tannins, anthocyanins, including cyanidin-, petunidin, malvidin-glucoside and other components[20]. The juice of unripe fruits is used for preparing vinegar that is considered to be a stomachic, carminative and diuretic. The ripe fruits are used for making preserves, squashes and jellies. The fruits are astringent. A wine is prepared from the ripe fruits in Goa. It is well known that its leaf extract can protect against radiation-induced DNA damage[21]. Extract of seed, which is traditionally used in diabetes, has a hypoglycemic action and antioxidant property in alloxan diabetic rats[22], possibly due to tannins. Fruit skin of *S. cumini* has significant antioxidant activity as previously reported[23, 24].

The present study aimed to evaluate the acaricidal ability of different extracts of *S. cumini* against *T. urticae*.

2. Materials and methods

2.1. Chemicals and plant materials

All chemicals and organic solvents were purchased from Sigma- Aldrich (St. Louis, MO).

2.2. Source of S. cumini

Mature fruits of *S. cumini* (pomposia) were collected at the campus of Faculty of Agriculture, Cairo University Giza, Egypt (Season July 2009) and identified by Dr. Shanan, Ornamental Department, Faculty of Agriculture, Cairo University. The fruits were ripened and freshly harvested.

2.3. Extraction of S. cumini

Fifty grams of plant fruits were subjected to extraction with different solvents according to Rossenthaler[25]. Hexane, petroleum ether (40-60), ethyl acetate, methylene chloride: methanol (1:1, v/v) and distilled water were used. The polarity was increased from non-polar to highly polar. Each solvent extract was evaporated under vacuum with rotary evaporator to dryness and then was weighed. Each extract was mixed with DMSO as emulsifier and prepared with different concentration of 75, 150 and 300 μg/mL.

2.4. Mites rearing

T. urticae was collected from infested cucumber plants (*Cucumis sativus* L.). Bean (*Phaseolus vulgaris* L.) seeds, and was kept in 14 cm diameter plastic blanks at a rate of 4-5 seeds per pot. Seedlings from this culture were infested with *T. urticae* adults. Adult mites were transferred to aluminum pans (30 cm×20 cm×70 cm) from fresh leaves of Beefsteak plant (*Acalypha wilkesiana* L.), and placed upside down on wet cotton pads. The emerged females and males were transferred to new Beefsteak plant for 2-3 days' culture and allowed to mate. Afterwards water was added when needed and kept in incubator at (25±2) ℃ and (70±5)% RH.

2.5. Mites treatment

Female *T. urticae*, 3 days old, were obtained by placing 100 duetonymphs from the culture, and on excised Beef steak leaves. They wewe then placed on wet cotton pads in Petri dishes. Forty females were transferred equally to four discs, 3 cm diameter, and then treated with one of the prepared extracts. Control treatment was operated by DMSO at a rate of 0.1%. Mortality was estimated for the adult females after 24 h of spraying and corrected by Abbot's formula[26]. LC_{50} of each extract was calculated according to Finney[27], then mites were treated with each extract at LC_{50} Survived mites were collected and used for enzyme determination.

2.6. Preparation of crude enzyme extracts from T. urticae

The method described by Vit-ria *et al*[28] was used to prepare the crude enzymes extracts. In this method, adults resistant *T. urticae* were mixed in a chilled pastel and mortar with 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrrolidone. The homogenates were centrifuged at 10 000 *g* for 30 min and then the supernatants were kept and stored in separate aliquots at -20℃ until analysis.

2.7. Antioxidant of enzyme activities

POD activity was assayed according to the method of Hemeda and Klein[29]. A total of 100 mL of reaction mixture containing 10 mL of 1% guaiacol (v/v), 10 mL of 0.3% H_2O_2 and 80 mL of 50 mM phosphate buffer (pH 6.6). Enzyme extract (75 μL) was added to reaction mixture in a final volume of 3 mL. The increase in absorbance due to oxidation of guaiacol (extinction coefficient 26.6 mM/cm) was monitored at 470 nm. Enzyme activity was expressed as unit's min/mg protein.

The activity of APX was measured by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM/cm). The 3 mL reaction mixture contained 50mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm[30] and enzyme activity was expressed as unit's min/mg protein.

For measurement of CAT activity, the method of Aebi [31] was used. A total of 3 mL reaction mixture comprised of 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and a suitable aliquot of enzyme. Decrease in the absorbance was taken at 240 nm (molar extinction coefficient of H_2O_2 was 0.04 mM/cm). Enzyme activity was expressed as units min/mg protein.

SOD activity was measured by the photochemical method as described by Beauchamp and Fridovich[32]. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. One unit of SOD is the amount of extract that gives 50% inhibition to the rate of NBT reduction.

2.8. Protein determination

Soluble protein was estimated by using the Coomassie Brillent Blue G-250 reagent according to the method of Bradford[33] with bovine serum albumin as standard.

2.9. Statistical analysis

All tests were conducted in triplicate. Data are reported as means ± standard error (SE). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package (Cohort Software, CA, USA). The least significant difference at $P<0.05$ level was calculated.

3. Results

The acaricidal activity of six different extracts of *S. cumini* (Pomposia) at concentrations of 75, 150 and 300 μg/mL against adult female of *T. urticae* were shown in Table 1. The results showed that, ethanol extract, had the most efficient

Table 1

Acaricidal activity of different solvent extracts from *S. cumini* against adult females of *T. urticae* (Mean ± SE)

Extract	Concentration (μg/mL)	Resistant	Mortality	LC_{50} (μg/mL)
Hexane	75	75.00 ± 0.28^a	25.00 ± 0.21^c	101
	150	25.00 ± 0.22^b	75.00 ± 0.33^b	
	300	5.89 ± 0.14^c	94.11 ± 0.40^a	
Chloroform	75	73.30 ± 0.34^a	26.70 ± 0.18^c	118
	150	40.00 ± 0.28^b	60.00 ± 0.31^b	
	300	33.40 ± 0.26^c	66.60 ± 0.39^a	
Ether	75	66.70 ± 0.18^a	33.30 ± 0.24^c	102
	150	35.00 ± 0.35^b	65.00 ± 0.41^b	
	300	10.00 ± 0.17^c	90.00 ± 0.65^a	
Ethyl acetate	75	68.75 ± 0.24^a	31.25 ± 0.29^c	98
	150	11.77 ± 0.16^b	88.23 ± 0.46^b	
	300	10.00 ± 0.14^c	90.00 ± 0.48^a	
Ethanol 70%	75	65.00 ± 0.27^a	35.00 ± 0.31^c	85
	150	15.00 ± 0.13^b	85.00 ± 0.64^b	
	300	0.00 ± 0.10^c	100.00 ± 0.00^a	
Water	75	63.16 ± 0.32^a	36.84 ± 0.29^c	120
	150	42.86 ± 0.41^b	57.14 ± 0.49^b	
	300	25.00 ± 0.26^c	75.00 ± 0.63^a	
DMSO (Control)	-	75.00 ± 0.23^a	15.00 ± 0.32^d	

Values are expressed as the means ± SE. Mean values with different letters in the same column were significantly different ($P < 0.05$).

Table 2
Effect of *S. cumini* on antioxidant enzyme activities (Mean ± SE).

Treatments	APX (Unit/mg protein)	POD (Unit/mg protein)	CAT (μ mol/mg protein/min)	SOD (Unit/mg protein)
Control	3.14 ± 0.06^{f}	5.23 ± 0.11^{g}	2.13 ± 0.06^{g}	1.67 ± 0.10^{g}
Hexane	3.96 ± 0.07^{f}	8.15 ± 0.11^{f}	4.31 ± 0.18^{f}	5.46 ± 0.07^{f}
Chloroform	5.31 ± 0.18^{e}	13.36 ± 0.36^{e}	8.55 ± 0.18^{e}	9.53 ± 0.11^{e}
Ether	7.81 ± 0.13^{d}	20.60 ± 0.20^{d}	10.37 ± 0.20^{d}	14.90 ± 0.18^{d}
Ethyl acetate	30.15 ± 1.02^{b}	46.84 ± 1.62^{b}	37.29 ± 0.98^{b}	26.67 ± 0.45^{b}
Ethanol	42.21 ± 0.94^{a}	60.38 ± 2.02^{a}	49.05 ± 0.99^{a}	35.87 ± 0.78^{a}
Water	16.3 ± 0.13^{c}	30.25 ± 1.91^{c}	28.41 ± 0.39^{c}	20.36 ± 0.23^{c}
LSD	0.983	2.14	0.981	0.636

SOD-Superoxide dismutase , CAT-Catalase , and APX-Ascorbate peroxidase, POD-Peroxidase.
Values are expressed as the means ±SE. Mean values with different letters in the same column were significantly different ($P < 0.05$).

acaricidal activity (100.00%) at concentration of 300 μg/mL followed by hexane extract (94.11%), ether (90.00%) and ethyl acetate extracts represent (90.00%). In contrast, adult females of *T. urticae* recorded the maximum resistant (33.40%) at concentration of 300 μg/mL of chloroform extract of *S. cumini* followed by water (25.00%), ether (10.00%) and ethyl acetate extracts(10.00%).

No previous studies have been reported on the acaricidal properties of *S. cumini* (Pomposia) solvent extracts against *T. urticae*. On the other hand antioxidants and anticancer activity of *S. cumini* (Pomposia) have been proved in our new publication (data not shown).

The efficiet fraction in ethanol extract was 8 120 mg/100g, followed by water extract (900 mg/100g), ether extract (70 mg/100g), ethyl acetate (50 mg/100g), ghloroform extract (30 mg/100g) and hexane extract (20 mg/100g).

The LC_{50} values after 24 h for ethanol extract, ethyl acetate extract, hexane extract, ether extract and water extract were 85, 98, 101 and 102 μg/mL, respectively. These results provided basis for further screening and separation of the acaricidal activity components from *S. cumini*, laid the foundation for the development and utilization of Egyptian abundant resources *S. cumini*, and brought important reference value for the mite pests' control of new natural acaricidal agents from *S. cumini* which considered important traditional Egyptian medicine plant. Our new investigation proved that the ethanol extract from *S. cumini* fruits have highest antioxidant and anticancer activity and this activity was correlated with the presence of different active ingredients especially γ-sitosterol and Kaempferol 7-O-methyl ether (data not shown).

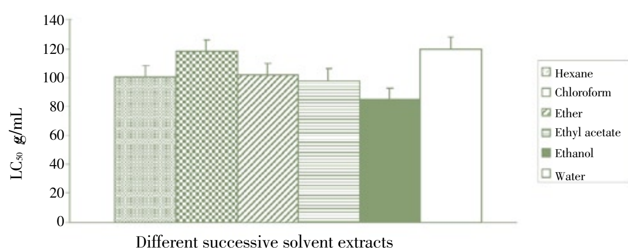

Figure 1. LC_{50} (μg/mL) of different successive extracts of *Syzgium* sp against *T. urticae*.

Table 2 showed that the activities of antioxidant enzymes APOX, POD, CAT and SOD of adult female *T. urtice* after treatments of *S. cumini* solvent extracts at LC_{50}. All enzymes activity significantly reached its maximum in ethanol extract followed by ethyl acetate, ether, chloroform and hexane, respectively. The increment of antioxidant enzymes may cause adult female *T. urtice* resistant against the treatments with *S. cumini* solvent extracts.

4. Discussion

In the present research, activities of antioxidant enzymes were detected in female adult *T. urtice* after treatments with different solvent extracts from *S. cumini*. However, no correlation between enzyme activity and their feeding habit was observed. Acaricidal activities of three essential oil extracts (Chamomile; Marjoram; and Eucalyptus) against *T. urticae* (Koch) have been approved and Chamomile is the most efficient one Afify AMR, *et al*[34]. Chamomile and Marjoram essential oil showed relationship between essential oil contents and activity of enzyme glutathione S-transferase, non specific esterase and alkaline phosphatase as well as inhibition of protease enzyme in *T. urticae*. The major essential oil contents of Chamomile are α-bisabolol oxide A (35.25%), and trans-β-farersene (7.98%), while the main components of Marjoram are terpinen-4-ol (23.86%), *P*-cymene (23.40%) and sabinene (10.90%). The major components of both plant extracts (Terpinen-4-ol 23.86% and α-bisabolol oxide A 35.25% may be responsible for the changes in enzyme activities of *T. urticae* (glutathione S-transferase, non specific esterase and alkaline phosphatase). It has been claimed that increased activities of detoxifying and antioxidative enzyme systems in arthropods may be positively correlate with polyphagy[35]. Such a correlation is comprehensible as organisms feeding on diverse diets are most likely

challenged by a variety of xenobiotics and prooxidant plant allelochemicals. Conceivably, such challenge has forced the development of mechanisms for survival and adaptation throughout evolution. Furthermore, induction of detoxifying enzymes by a large number of toxicants has been observed in arthropods[36-40].

Stress induces several physiological, biochemical and molecular responses in several crop plants, which would help them to adapt to such limiting environmental conditions. It inhibits the photosynthesis of plants, causes changes of chlorophyll contents and components and damage to the photosynthetic apparatus. It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle. The alteration of antioxidant metabolisms is one of the fundamental metabolic processes that may influence the drought tolerance of perennial grasses. A common effect of drought stress is the disturbance between the generation and quenching of re-active oxygen species [40].

The significant increase of antioxidant enzyme activities such as APX, POD, SOD and CAT, may be driven by enhancing thiobarbituric acid reactive substances, ROS and H_2O_2 formation in adult female *T. urticae* by solvent extracts treatments from *S. cumini*. Oxidative burst has shown inducing effect on the expression of a variety of defense genes[36]. To evade the potential damaging effects of ROS, cells have evolved protection mechanisms, including antioxidant enzymes, such as SOD, CAT, and APX or guaiacol peroxides (GPOD)[35]. SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT, POD and/or APX to water and oxygen. The antioxidant enzymes' activities play an important role in scavenging ROS and therefore their improvement could increase the ability to tolerate ROS stress and delay its senescence. These findings suggest that genes encoding antioxidative enzymes can be activated by the increased production of active oxygen derivatives in adult female *T. urticae*. It is possible that these antioxidative processes protect *T. urticae* from lipid peroxidation processes around the infection sites and thereby protect them from the solvent extracts treatments from *S. cumini*[36]. Such a correlation is comprehensible as organisms feeding on natural extracts are most likely challenged by a variety of xenobiotics and pro-oxidant plant allelochemicals. Conceivably, such challenge has forced the development of mechanisms for survival and adaptation throughout evolution. Furthermore, induction of detoxifying enzymes by a large number of toxicants has been observed in arthropods.

As a consequence, plants' evolved cellular adaptive responses like up-regulation of oxidative stress protectors and accumulation of protective solutes. Antioxidant defense enzymes such as superoxide distumase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) are the systems designed to minimize the concentrations of superoxide and hydrogen peroxide. Superoxide-dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. (H_2O_2) is eliminated by catalase and peroxidases, which include both enzymic and non-enzymic H_2O_2 degradation. The antioxidants such as ascorbate (AsA) and glutathione (GSH) are involved in scavenging ROS primarily by the Halliwell-Asada pathway, which scavenges H_2O_2, while MDAR and GR are involved in the regeneration of ascorbate[39-40].

Ethanol extract of *S. cumini* (Pomposia), has the most efficient acaricidal activity against *T. urticae* followed by hexane extract, ether and ethyl acetate extracts. The activities of antioxidant enzymes APX, POD, SOD, CAT in susceptible mites were reach the maximum values with ethanol and ethyl acetate extracts at LC_{50} respectively.

Conflict of interest statement

We declare that we have no conflict of interest.

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