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

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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,

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 LC₅₀ values of the promising extract were 85.0, 101.0, 102.0 and $98.0 \,\mu$ 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_{so} 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.

> 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 nonenzymatic 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 (OH) and singlet oxygen (¹O₂), 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 prooxidant 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 μ g/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) $^{\circ}$ 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 were 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]. LC₅₀ of each extract was calculated according to Finney^[27], then mites were treated with each extract at LC₅₀ Survived mites were collected and used for enzyme determination.

2.6. Preparation of crude enzyme extracts from T. urticae

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