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Antifungal activity of Tithonia rotundifolia Blake and Tagetes erecta L against Aspergillus niger

[1]Ruby Patel, [2]Jahnavi Pandya, [2]Nikisha Rajpurohit, [3]Ranna Chaudhary and [4]Hetal Bhalakiya

[1]Assistant Professor, M. N. College, Visnagar, Gujarat, India.

[2,4]Ph.D. Student, Department of Botany, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

[3]Ph.D. Student, Seth M. N. College, Patan, Gujarat, India.

Abstract:

Aspergillus niger fungus is one of the most widespread species of the genus Aspergillus. This fungus includes a set of fungi that are usually considered asexual, although perfect forms that reproduce sexually have been found. Because of its cosmopolitan nature, human beings get commonly exposed to spores and vegetative forms of *A. niger* present in the air, on foodstuffs and others stored consumables products and suffers with many allergic problems. This fungus also causes disease in plants, *i.e.* rot diseases. Pathogenic fungi are the major infectious agents in plants which causes alterations during developmental stages of life, including post-harvest The antifungal properties of horticultural crops *Tithonia rotundifolia* Blake and *Tagetes erecta* L on rot fungal pathogen *Aspergillus niger* are therefore aiming of present work. This is to serve as a relative alternative to the use of synthetic chemicals to decrease or eliminate loss due to rot caused by phytopathogens chiefly fungi. The conclusion of this experiment definite that plant extracts can be used as natural fungi toxicant to control the growth of pathogenic fungi (*A. niger*) and consequently reduce the dependence on the synthetic fungicides.

Keywords: *Aspergillus niger,* antifungal, *Tithonia rotundifolia* Blake and *Tagetes erecta* L.

Introduction:

One of the most common species of the genus Aspergillus is *Aspergillus niger* which includes a set of fungi that are commonly considered asexual, although perfect forms that reproduce sexually. On certain fruits like grapes and vegetables such as onions and peanuts it causes a disease called black mold. It is a common contaminant of food. Naturally Aspergilli are ubiquitous. They are geographically broadly distributed and have been observed in a broad variety of habitats because they can colonize a large variety of substrates. Aspergillus niger (black mold), a filamentous ascomycete having capability of rapid growth and pH tolerance is most significant cosmopolitan fungi related to postharvest decay of diverse substrates [1-3]. It is generally found as a saprophyte growing on stored grain, compost piles, dead leaves and other decaying vegetation. Because of its cosmopolitan nature, human beings get habitually exposed to spores and

vegetative forms of *A. niger* present on foodstuffs, in the air and others stored consumable products which cause allergic problems. Certain mycotoxins produce by this fungus which are nephrogenic, heptocarcinogenic and immunological in nature. Rot diseases caused by this fungus.

Pathogenic fungi are the chief infectious agents in plants which cause alterations during developmental stages of life including post-harvest. In vegetables and fruits, there is a broad variety of fungal genera causing quality problems related to an aspect, organoleptic characteristics, nutritional value and partial shelf life [4]. Chemical control is valuable and efficient, but, at the same time it can lead to the development of pathogen resistance and chemical residues in plant part including phytotoxicity to other organisms or public health and problems environmental [5-6].

Previous reports [7-8] illustrate that herbs, spices and other plant materials acquire antifungal activity. The antifungal properties of horticultural crops *Tithonia rotundifolia* Blake and *Tagetes erecta* L on rot fungal pathogen, *Aspergillus niger* are therefore aimed at in this finding. This research serves as a relative option to the use of synthetic chemicals to eliminate loss or reduce due to rot caused by phytopathogens chiefly fungi and the resultant economic loss to the consumers, traders and farmers.

Material and Method:

Plant material: Plant material was collected from Anand Agricultural University. Different plant part i.e. leaf and flower from *Tithonia rotundifolia* Blake and *Tagetes erecta* L plant used in traditional medicine. The healthy leaves and fully opened flowers were shade dried and minced to a fine powder in order to be used for the extraction.

Preparation of plant extracts: The method was given by [9] which were adopted for preparation of plant extracts with slight modifications. In brief, 420 g portions of the powdered plant material were soaked separately in 100 ml of hexane and hydro alcohol (50% v/v) for 72 h. After every 24 h each mixture was stirred using a sterile glass rod. Each extract was passed through Whatmann filter paper no1 (Whatmann, England) at the end of extraction. The filtrate extract was obtained and concentrated *in vacuo* using rotary evaporator at 30 ºC.

Culture Media: Sabouraud's dextrose agar/broth media was used for antifungal test (Hi media Pvt. Bombay, India).

Inoculum: The fungal strains were inoculated individually in Sabouraud's dextrose broth for 6 h. The suspensions were checked to offer around 10^5 CFU/ml.

Purpose of antifungal activity: The agar well diffusion method by [10] was adapted. For fungal cultures Sabouraud's dextrose agar (SDA) was used. The culture medium was inoculated with the fungal strains and then separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and then filled with plant extracts and solvent blanks (hydro alcohol, and hexane). As a positive

control Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used. Fungal plates were incubated at 37ºC for 72 h. The diameters of zone of inhibition observed were measured.

Determination of MIC and MBC: The antifungal plant extracts were assessed to determine MIC and MLC values. The broth dilution method was adopted by using N-saline for diluting the plant extract and then incubated for 48 h. The minimum dilution of the plant extract which kills the fungal growth was taken as MLC (Minimum lethal count) whereas the minimum dilution of plant extract which inhibits the growth of the organism was taken as MIC

Result:

All the extract obtained from the selected plants presented antifungal potential against the tested fungus, demonstrated by the inhibition of the fungal mycelia growth. In general alcoholic extract showed more intense antifungal activity than the hexane solution. The results revealed that the antifungal activity of the plant extract was dependent on dose, being negatively influenced by the decreasing the concentration of the extract in the growth media.

Hydroalcoholic extract of *Tithonia rotundifolia* Blake flowers possessed potent antifungal activity amongst all the all extracts of plants against *Aspergillus niger* showing 20 mm diameter of zone of inhibition while hydroalcoholic extracts of *Tagetes erecta* L flower showed 15 mm diameter of zone of inhibition. Hexane extracts of *Tagetes erecta* L flower showed antifungal activity against *Aspergillus niger*, showed 13 mm diameter of zone of inhibition while *Tithonia rotundifolia* Blake flowers showed diameter of zone of inhibition viz. 17 mm.

A Hydroalcoholic extract of *Tithonia rotundifolia* Blake leaves showed more antifungal activity as compare to the *Tagetes erecta* L leaves viz. 15 mm. while hydroalcoholic extracts of *Tagetes erecta* L flower showed diameter of zone of inhibition viz. 10 mm. Hexane extracts of *Tagetes erecta* L leaves showed antifungal activity against *Aspergillus niger*, diameter of zone of inhibition viz. 09 mm while *Tithonia rotundifolia* Blake leaves showed more antifungal activity i.e. diameter of zone of inhibition viz. 17 mm.

Table-1: Antifungal activity of two plant extracts

Sr. No.	Plant	Plant Part	Solvent Extract	Diameter of Zone of Inhibition (mm)	MIC (mg/ml)	MLC (mg/ml)
1.	*Tagetes erecta* L	Flower	HA	15	0.5	0.6
			HX	13	0.7	0.8
		Leaf	HA	10	0.4	0.5
			HX	09	0.6	0.7
2.	Tithonia rotundifolia Blake	Flower	HA	20	0.5	0.6
			HX	17	0.6	0.8
		Leaf	HA	15	0.5	0.6
			HX	14	0.6	0.7

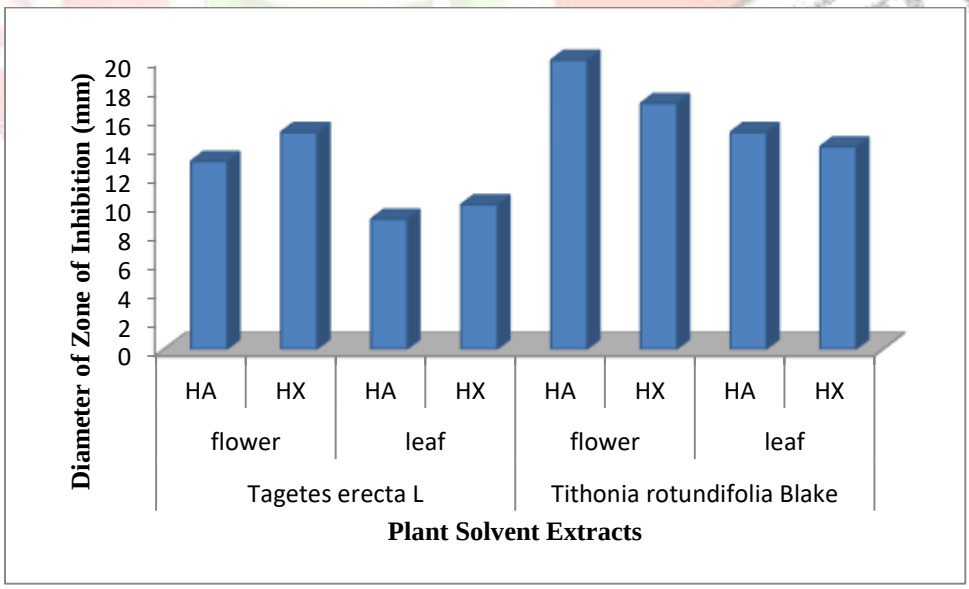

Fig-1: Antifungal activity of plant solvent extracts

Discussion: Commonly, synthetic fungicides controlled phytopathogenic fungi; yet, the use of these is more and more limited due to the harmful effects of pesticides on the environment and human health [11]. Plants might characterize another treatment in non-severe cases of infectious diseases. Some Indian plants were

reported for Antioxidant, Antimicrobial and Anti-inflammatory properties [12]. Banso & Adeyemo [13] supports the present findings.

Numerous works have established in laboratory trials that different plant tissues, such as seeds, leaves, roots and flowers acquire inhibitory properties against bacteria, fungi and insects [14]. The antifungal efficacy of leaf and stem extract of *Mirabilis jalapa* reduce mycelial growth of four different strains of fungi have reported [15]. The use of local plant extracts for the protection of mechanically injured sweet potato has reported [16]. In present study Eugenol in clove and cimnamelaldehyde in cinnamon is reported as the chief active compound, which might be liable for their antifungal potential against A. niger [17]. Earlier the antifungal effect of S. aromaticum on Aspergillus spp. and C. zeylanicum found on Penicillium spp. was also reported [18-20].

Conclusion: Hydroalcoholic and hexane extracts of leaves and flowers of *Tagetes erecta* L and *Tithonia rotundifolia* Blake is found effective against the growth of test organism. Therefore, this study suggests that the hydroalcoholic and hexane extracts of these species would be helpful in treating diseases in plants caused by *A. niger*. In conclusion, the result of this experiment confirmed that Hydroalcoholic and hexane extracts of leaves and flowers of *Tagetes erecta* L and *Tithonia rotundifolia* Blake can be used as natural fungitoxicant to control the growth of pathogenic fungi (*A. niger*). Thus it reduces the dependence on the synthetic fungicides.

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