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Shricharan S
Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Mahalakshmi S
Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Kaviraj S
Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Corresponding Author:
Shricharan S
Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

In vitro evaluation of efficacy of botanicals against *Aspergillus niger* causing collar rot in groundnut

Shricharan S, Mahalakshmi S and Kaviraj S

Abstract
In view of organic production, botanicals play a significant role in management. Ground nut, an important oil seed crop is affected by various plant pathogens. Among this *Aspergillus niger* causes collar rot in groundnut. Four plant extracts were tested against *Aspergillus niger* under four different concentrations i,e 5, 10,15 and 20%. The study was done in vitro by poison food technique. Out of four plant extracts, maximum inhibition was found in garlic (80.00) at 20% followed by tulsi (71.87), green chilli (69.23) and ginger (62.68). The least mean inhibition was found in garlic (62.68). It was found that inhibitory effect increases with increase in concentration of plant extracts.

Keywords: Botanicals, collar rot, inhibition, mycellial growth and concentrations

1. Introduction
Groundnut (*Arachis hypogea*) is a tropical legume and one of the major oilseed crops. Groundnut is a native crop of South America (Gregory *et al*., 1976) [4]. The term Groundnut is derived from two Greek words *"Arachis*" means legume and "*hypogea" means* below the ground. Differing from other plant genera, this genus produces fruits below the ground but flowers, leaves and stem form above ground (Krapouickas *et al*., 1994) [5]. It is also called as 'King' of oilseeds, wonder nut, poor men's cashew nut. Groundnut is grown on 26.4 million ha worldwide with a total production of 37.1 million mt/ha and an average productivity of 1.4 metric t/ha (GOI., 2008) [3]. Developing countries constitute 97% of the global area and 94% of the global production of this crop. It is not only used for oil production, but also for human consumption and animal consumptions in the form of hay, silage and cake. The crop is the second most cultivated food legume and the fourth largest edible oilseed crop in the world (Shilman *et al*., 2011) [13]. The seeds have palmitic, oleic and linoleic acid accounting for about 90% of total fatty acids at seed maturity (Sekhon *et al.,* 1972) [12].
Peanut crop is susceptible to various diseases like Early leaf spot, Late leaf spot, Rust, Stem blight, Bud necrosis, *Alternaria*. Among the diseases that occur during seed and seedling stages, pre-emergence seed rot and post-emergence collar rot caused by *A. niger* is very important. Collar rot disease on groundnut seedlings was first reported by Jochem (1926) and in India it was first reported by Jain and Nema (1952) as *Aspergillus* blight. Collar rot caused by *A. niger* is a widespread disease, which causes rotting of seed, pre-emergence soft rot of hypocotyls and post-emergence collar rot of seedlings (Mehan *et al.,* 1995) [9]. Occasionally, collar rot can continue upto crop harvesting stage resulting in damage to the seed (Gajera *et al.,* 2011) [2]. This disease cause severe seedling mortality resulting in patchy crop stand mostly in sandy loamy soils. The loss due to this disease was reported 28 to 50% (Bakhetia *et al.,* 1983) [1]. *Aspergillus niger* is a saprophyte, filamentous fungus having smooth walled, hyaline conidiophores. Conidia are glosbose to sub globose (1.38micrometer-4micrometer diameter), dark brown to black and rough walled (Kumari *et al.,* 2017) [6]. *Aspergillus niger* was the most toxic fungus and causing yellowing of the leaves, blighting effect on the shoot part and at last finally leading to death of crown portion of the plant (Suzui *et at.,* 1980) [14]. The favourable conditions for the disease development includes deep sowing of seeds, high soil temperature (30-35 °C) and low soil moisture. The pathogen survives in soil, plant debris. The primary source of infection is soil-borne conidia whereas secondary sources are infected seeds. The pathogen is also seed borne in nature. Soil borne diseases are complicated to manage due to the difficulty of dispersing fungicide through the peanut canopy to the soil profile. And also continous fungicide applications results in depletion of beneficial rhizosphere microorganisms. Therefore, the present investigation was done about antifungal activity of few botanical extract (Tulsi, Ginger, Garlic, Green chilli) which was assayed *in vitro* by Poison food technique against *Aspergillus niger*.

2. Materials and Methods

2.1 Isolation and purification of fungi

Soil samples were collected from collar rot infected field and brought to the laboratory. The fungi was enumerated on Rose Bengal Agar (RBA). 1g of soil sample was serially diluted to 10^{-4} times. Standard procedures of pour plate method were followed. 1ml of the sample from 10^{-4} dilution were added in sterilized RBA in petri plates. The plates were allowed to solidify and incubated at 25±1 ºC. after 72 hrs of incubation the fungi were purified by disc transfer method on Potato Dextrose Agar (PDA) and stored for the further work. The microscopic observations with Lactophenol Cotton Blue staining (LPCB) were done and colony morphology were also taken in account.

2.2 Preparation of botanical extracts

The experiment were carried out to test the toxicity of four plant extracts (table 1) against *Aspergillus niger*. 50g of plant part were collected and washed with distilled water and air dried completely. The plant parts were crushed separately with 50ml of sterile water. The extract was filtered and centrifuged at 6000rpm for 10 minutes and the supernated were collected. The extract of each plant was diluted in order to achieve concentration of 5, 10, 15 and 20%.

Table 1: Botanicals and parts tested against *Aspergillus niger*.

S.no	Common name	Botanical name	Plant parts used	Concentration
1.	Tulsi	*Ocimum tenuiflorum*	Leaf	5, 10, 15, 20
2.	Ginger	*Zingiber officinale*	Rhizome	5, 10, 15, 20
3.	Garlic	*Allium sativum*	Clove	5, 10, 15, 20
4.	Green chilli	*Capsicum annuum*	Fruit	5, 10, 15, 20

2.3 Efficacy of plant extracts against pathogen

The growth of *Aspergillus niger* in various concentration of plant extracts were tested by standard procedure of poison food technique (Mayer, 1962) [8]. The Potato Dextrose Agar (PDA) was prepared and sterilized. The botanical extract of each concentration and PDA medium was mixed in equall volume and poured in sterile petri plates aseptically. The plates were left to solidify. 5mm of the fungal disc were transferred centrally on PDA plate. A suitable control without plant extract were maintained. The diameter of the fungal colony was measured after 5 days of incubation at 25±1 ºC. The percent mycelial growth inhibition was calculated by formula (Vincent's 1947) [15].

Percent mycellial growth inhibition = C - T / C x 100

Where,

C = Diameter of the colony in control.

T = Diameter of the colony in treatment.

3. Result and Discussion

3.1 Isolation and purification of fungi

The fungi was isolated from collar rot infected soil. The colony was initially white and spore developed after 3 days. The fungi was purified by disc transfer method. The fungi covered the entire plate after 7 days of incubation. The colony and microscopic observation (Fig.1) is given in table 2

Table 2: Colony morphology and Microscopic observation of fungi

S. No	Characteristics	Observations
1.	Color of mycelium	Yellow white
2.	Spore formation	After 3 days
3.	Margins	Entire
4.	Size of colony (mm) a. After 3 days b. After 5 days c. After 7 days	 24.00 39.00 58.00
5.	Hyphae	Branched septate
6.	Cleistothecia	Present

3.2 Efficacy of botanicals against *Aspergillus niger*

Four plant extracts were tested in vitro against the collar rot pathogen. The extracts were diluted to 5, 10, 15 and 20% concentration with distilled water. The fungal growth was measured and recorded after five days of incubation. (Table 3) it was found that garlic extract produced maximum inhibition (80.00%) of mycellial growth, followed by tulsi (77.05%), green chilli (73.81%) and ginger (67.63) for 20% respectively (Fig.2). The mean mycellial inhibition was recorded highest in garlic (75.07%) and least in ginger (62.68%). Similar results were also observed by Manju kumari (2015) [7] on working with *A. niger* fungi.

Table 3: Efficacy of botanicals against A niger under different concentrations

S. No	Botanicals	Parts used	Percent inhibition of mycelium				Mean
			5%	10%	15%	20%	
1.	Tulsi	Leaf	67.11	69.97	78.15	77.05	71.87
2.	Ginger	Rhizome	57.98	61.24	63.89	67.63	62.68
3.	Garlic	Clove	69.50	72.63	78.15	80.00	75.07
4.	Green chilli	Fruit	64.41	67.22	71.50	73.81	69.23

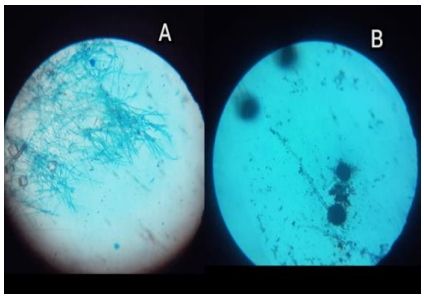

Fig 1: LPCB staining of *A. niger* A. mycelium and B. sporangium.

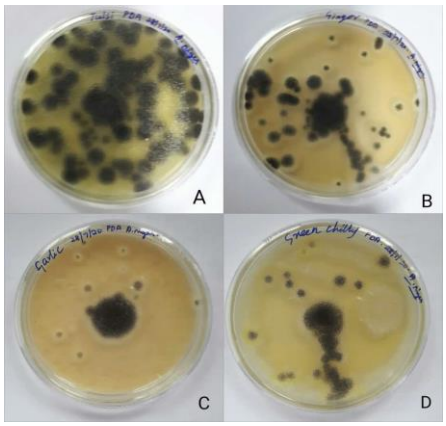

Fig 2: Mycellial growth on 20% concentration of A. Tulsi extract. B. Ginger extract. C. Garlic extract. D. Green chilli extract.

Within all four plant extacts, all four levels of extracts i.e, 5, 10, 15 and 20% showed significant difference from each other. It was also found that inhibition of the mycellial growth was maximum in higher concentration. Leaf extracts like turmeric, onion, tulsi and coleus were found to be effective against spoilage organism *Aspergillus* spp. reported by Roopa. V *et al.*, 2014 [10].

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