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IN VITRO CONTROL OF FIVE PATHOGENIC FUNGI ISOLATED FROM GROUNDNUT (ARACHIS HYPOGAEA L.)

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Abstract

Efficacy of five plant extracts namely, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Tagetes patula* and *Zingiber officinale* was evaluated against five pathogenic species of fungi isolated from groundnut *in vitro*. These were *Colletotrichum acutatum*, *Colletotrichum dematium*, *Colletotrichum orbiculare*, *Colletotrichum* sp. and *Fusarium semitectum.* Colony growth of *C. dematium* was completely checked with *Allium sativum* at all the concentrations used (5, 10 and 20 %). Similarly *A. cepa*, *A. sativum* and *A. indica* completely inhibited the colony growth of *C. orbiculare* at the same concentrations used. *T. patula* and *Z. officinale* also showed appreciable inhibition in colony growth of five species of fungi at 10 and 20% concentrations.

Key words: *In vitro*, Control, Pathogenic fungi, Groundnut

Introduction

Groundnut, one of the principal economic crop of world occupies 13th position among fruit crops (Varnell and Mccloud 1975), 4th place among the oilseed crops in respect to both area and production next to soybean, sunflower and cotton (Weiss 1983). Groundnut is the second major oil crops in Bangladesh covering an area of 76 thousand ha. producing 1.2 million MT of nuts. Bangladesh produces 46000 MT of groundnut (BBS 2007). Increase in the production of this crop can help to minimize the shortage of edible oil in our country. It is the richest plant source of thiamin (B_1). Groundnut contains at least 13 different types of vitamins and also rich in 26 essential minerals. Incidence of disease is the most important obstacle for groundnut production. Fungi can be rendered as the most harmful microorganism and so far, 46 fungal diseases were recorded on groundnut and 67 (aprox.) fungi were associated with various symptoms type (Wikipedia 2012). In Bangladesh, groundnut suffers from many diseases out of which 14 are fungal, two are viral, nine are nemic and one is mycoplasma disease (Ahmed 1985, Baker *et al.* 1980, Fakir 1980 and Talukder 1974). Shamsi and Sharmin (2012) recorded ten types of symptoms on eighteen varieties of groundnut during the period of December 2010 to May 2012. This investigation also revealed that a total of 48 species of fungi representing 24 genera was associated with 18 vareites of *Arachis hypogaea*. To protect groundnut from diseases, one must have knowledge on etiology of disease, isolation and identification of causal organism, prevention and control measure. Much research works have been carried out on management of diseases of groundnut in different parts of the

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world (Ambang *et. al*. 2011, Sing *et al.* 1997 and Sunker *et al.* 2005). In Bangladesh very little work has been done to protect groundnut from the incidence of diseases (Mia *et al.* 2007, Bakr *et al*. 2009 and Sharmin 2012). Control of plant diseases by using plant extract having antifungal properties has recently gaining appreciable importance to plant pathologists. Intensive research has been done in this field to avoid the hazardous impact of pesticides and agro-chemicals on ecosystem. On account of their non phytotoxicity, biodegradability and renewable nature such substances appear to be the ideal antifungal agents (Baker *et al.* 1980). Present study was undertaken to (i) find out the association of the fungi with groundnut (ii) determine the pathogenic potentiality of the fungi and (iii) evaluate antifungal potentiality of some botanicals *in-vitro* against most frequently isolated fungi from groundnut.

Materials and Methods

Collection of samples: During the period of December 2010 to May 2012, 18 varieties of groundnut plant were grown in field plot of Botanical garden, Curzon Hall, University of Dhaka. Samples were collected from Botanic Garden (Curzon Hall campus), University of Dhaka and BARI, Gazipur. Collected samples were examined and symptoms were recorded. After microscopic observation fungi were isolated from healthy and diseased samples following the Blotter" and"Tissue planting" method on PDA medium (CAB 1968). Specimens were preserved in the Herbarium of Mycology and Plant Pathology laboratory, Department of Botany, University of Dhaka. The varieties used in the experiment were: GN, BB- 8, DG- 2, B- 5, B- 6, B- 7, BN- 1, BN- 2, BN- 3, BN- 4, DHAKA- 1, BARI- 5, BARI- 6, BARI- 7, BARI- 8, BARI- 9, GN_1 and GN_2. Isolated fungi were tested for their pathogenic potentiality. In the present investigation 48 species of fungi were isolated from groundnut and identified following Standard Literature (Booth 1971, Ellis 1971, 1976, Sutton 1980, Ellis and Ellis 1997, Barnett and Hunter 2000). Isolated fungi were tested for their pathogenic potentiality following modified "detached leaf technique" (Azad and Shamsi 2011). *Cercospora arachidicola* S. Hora, *Pheoisariopsis personata* Berk and M.A., *Puccinia arachidis* Speg. and *Sclerotium rolfsii* are well documented pathogens of groundnut. In this experiment five fungi namely *C. acutatum* Simmonds*, C. dematium* (Pers. Ex. Fr.) Grove., *C. dematium orbiculare* (Berk. & Mont.) Arx., *Colletotrichum* $sp._1$ and *Fusarium semitectum* Berk. & Rav. were found to be pathogenic to groundnut. Efficacy of five plant extracts namely *Allium cepa* L.*, A. sativum* L.*, Azadiracta indica* L.*, Tagetes patula* L. and *Zingiber officinale* L. was evaluated against these five pathogenic fungi following poison food techniques (Grove and Moore 1962).

Preparation of plant extracts: The desired parts of each plant were thoroughly washed in tap water, air dried and then used for fresh extract preparation (Table 1). In case of leaves and bulbs, extracts were prepared by crushing known weight of fresh materials with distilled water in ratio of 1:1 (w/v). The pulverized mass of a plant part was

squeezed through four folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 minutes to remove particulate matter. The supernatants were filtered through Whitman filter paper and the filtrate was collected in 250 ml Erlenmeyer flasks. In this method, the requisite amount of the filtrate of each plant extract was mixed with PDA medium and sterilized in an autoclave at 121°C for 15 minutes.

Table 1. The particulars of plant extracts used in this experiments.

Plant species	Native name	Family	Plant part used
Allium cepa	Onion	Liliaceae	Bulb
Allium sativum	Garlic	Liliaceae	Bulb
Azadiracta indica	Neem	Meliaceae	Leaf
Tagetes patula	Marigold	Asteraceae	Leaf
Zingiber officinale	Ginger	Zingiberaceae	Rhizome

The medium thus prepared was poured into sterilized Petri plates and was allowed to solidify. Each Petri plates was inoculated centrally with a 5 mm agar disc cut from the margin of actively growing culture of the test pathogen. In control set, a Petri plate containing PDA medium with the requisite amount of distilled water instead of a plant extract was also inoculated with agar disc of the test pathogen in the same way as described above. Three replications were maintained for both treatment and control sets. The inoculated Petri plates were incubated at 25 ± 1°C. The radial growth of the colonies was measured after 5 days of incubation.

The percentage growth inhibition of each test pathogen was calculated by using the following formula

$$I = \frac{C - T}{C} \times 100$$

Where, I = percent growth inhibition, C = growth in control and T = growth in treatment

Results and Discussion

Use of plant extracts against plant pathogenic fungi and plant diseases is relatively a recent approach. Five fungi, isolated from leaflets of groundnut showing anthracnose, Colletotrichum leaf spot and rotting symptom were found to be pathogenic to the plant. This is the first report of anthracnose, Colletotrichum leaf spots caused by *Colletotrichum* spp. from Bangladesh. The isolated fungi were *Colletotrichum acutatum, C. dematium, C. orbiculare, Colletotrichum* sp. and *Fusarium semitectum.* Efficacy of five plant extracts namely *Allium cepa, A. sativum, Azadiracta indica, Tagetes patula* and *Zingiber officinale* was evaluated against those five fungi. The extent of inhibition, however, varied among the fungi.

The vegetative growth of *C. acutatum* showed highest (69 %) inhibition with *T. patula* at 20 % followed by 53 and 46 % inhibition at 10 and 5 % concentration respectively. *Z. officinale* showed 36 % inhibition of the fungus at highest concentration (20 %). Lowest

inhibition of the fungus such as 20, 14 and 11 % was encountered at 20, 10 and 5 % concentrations respectively (Fig. 1). This observations are in agreement with the observations made by Sing *et al.* (1997) and Mala *et al*. (1998).

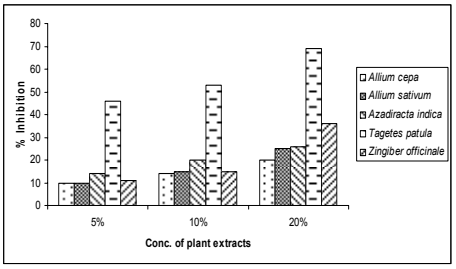

Fig.1. Effect of plant extracts on growth of *Colletotrichum acutatum.*

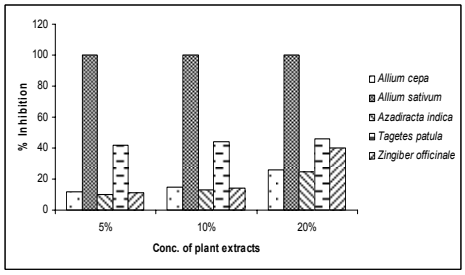

Fig. 2. Effect of plant extracts on growth of *Colletotrichum dematium*.

Colletotrichum dematium was completely checked with *A. sativum* at all the concentrations used (5, 10 and 20 %). Less inhibition of the fungus was recorded when treated with 10, 13 and 25 % of *A. indica* at 5, 10 and 20 % concentrations (Fig. 2). Higher fungitoxicity of *A. sativum* was also reported by Misra and Dixit (1976). Seed borne pathogens of jute were effectively controlled by *Allium sativum* (Ahmed and Sultana 1984). Shovan *et.al.* (2008) recorded 89.44 % inhibition of *C. dematium* isolated from anthracnose of Soybean.

The growth of *C. orbiculare* was completely inhiteded by *A. cepa*, *A. sativum* and *A. indica* at all the concentrations used (5, 10 and 20 %). *Z. officinale* and *T. patula* checked 45 and 34 % colony growth of the fungus respectively at 20 % concentration (Fig. 3).

Colonies of *Colletotrichum* sp. was 57 % inhibited *by Z. officinale* at 20 % concentration followed by *T. patula*, 51 % inhibition of the colony at the same concentration. *A. cepa* and *A. indica* showed 39 % inhibition of the fungus at 20 % concentration. *A. sativum* inhibited 30, 36 and 45 % colony growth of the fungus at 5, 10 and 20 % concentrations respectively (Fig. 4).

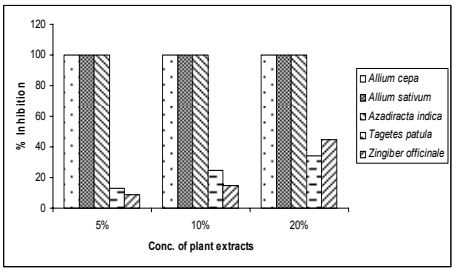

Fig. 3. Effect of plant extracts on growth of *Colletotrichum orbiculare.*

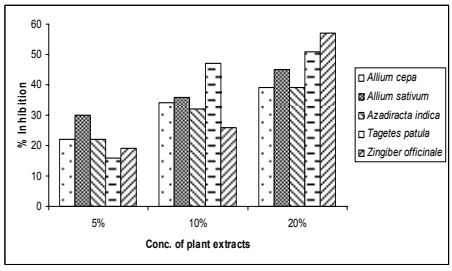

Fig. 4. Effect of plant extracts on growth of *Colletotrichum* sp.

Plant extract of *T. patula* inhibited 81 % vegetative growth of *Fusarium semitectum* at 20 % concentration followed by 62 and 33 % at 10 and 5 % concentration respectively. *Z. officinale* checked 71, 61 and 42 % colony growth of the fungus at 20, 10 and 5 % concentrations respectively. *A. sativum* showed 62, 44 and 38 % inhibition of the fungal colony at the above mentioned concentrations. *Allium cepa* inhibited 53, 41 and 34 % colony growth of the fungus at 20, 10 and 5 % concentrations respectively. *A. indica* showed lowest inhibition of the fungus 49. 43 and 20 % at 20, 10 and 5 % concentrations. Methanol extract of *T. patula* inhibited growth of three pathogenic fungi *Botrytis cineria*, *Fusarium moniliformae* and *Phythium ultimum* (Mares *et al.* 2004) (Fig. 5).

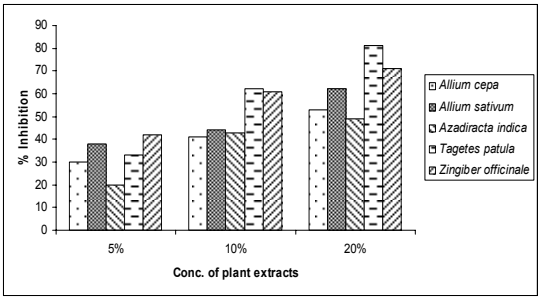

Fig. 5. Effect of plant extracts on growth of *Fusarium semitectum.*

In home and abroad, cultural practice and chemical control have been practiced against leaf spot, rust and stem rot diseases of groundnut, but this is the first approach of controlling the causal agents of anthracnose, Colletotrichum leaf spot and leaf rot of groundnut with botanicals *in vitro* .

Allium sativum completely inhibited the growth of *C. dematium* at all the concentration used (5, 10 and 20 %). *Allium cepa*, *A. sativum* and *A. indica* completely inhibited the growth of *C. orbiculare* at the same concentration used (5, 10 and 20%). *T. patula* and *Z. officinale* also showed appreciable inhibition in colony growth of five species of fungi at 10 and 20 % concentrations. Among the five plant extracts used *A. cepa*, *A. sativum* and *A. indica* showed excellent results in controlling the redial diameter of the colonies of *C. orbiculare* at 5 % concentration. In addition to *C. orbiculare, A. sativum* also exhibited complete inhibition of *C. dematium* at the same concentration. Hadian (2012) reported that *Allium sativum* and *A. indica* at 100% concentrations inhibited growth of *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* two pathogenic fungi causing wilting disease of tomato.

This is the first report of evaluation of plant extracts against *Colletotrichum* spp. and *F. semitectum* isolated from groundnut. The present findings on the antifungal activities of these plant extracts may, in future, open a new horizon in plant disease control.

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