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IN VITRO EVALUATION OF FUNGICIDES AND PLANT EXTRACTS AGAINST THE FUNGI ASSOCIATED WITH SEEDS OF NINE CHICKPEA VARIETIES

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Abstract

A total of nine species of fungi were found to be associated with seeds of nine varieties of chickpea. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link., *A. niger* Van Tiegh., *A. fumigatus* Fresenius., *A. nidulans* Eidam, *Curvularia lunata* (Wakker) Boedijn, *Penicillium* Link., *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers. Five fungicides *viz.*, Bavistin 50 WP, Dithane M-45, Greengel 72 WP, Ridomil MZ Gold and Tall 25 EC at 100, 200, 300, 400 and 500 ppm were evaluated against the test fungi. Tall 25 EC were found most efficient inhibitor which completely inhibited the radial growth of the test fungi followed by Bavistin 50 WP, Dithane M-45 and Greengel 72 WP. Antifungal properties of ethanol extracts of *Azadirachta indica* A. Juss., *Allium sativum* L., *Citrus medica* L., *Datura metel* L. and *Psidium guajava* L. at 5, 10 and 20% concentrations were evaluated against the test fungi. *Allium sativum* L. was found most efficient inhibitor of the test fungi followed by *D. metel* L., *A. indica* A. Juss., *C. medica* L. and *P. guajava* L.

Introduction

Chickpea (*Cicer arietinum* L.) belongs to the family Fabaceae is one of the important pulse crops grown in Bangladesh. It is an important protein rich crop and occupied third position both in production and in acreage in pulse production of Bangladesh. Some major constraints have sharply declined its cultivation area and production for the last several years[1]. Chickpea is invaded by more than 50 diseases reported from different parts of the world [2-4]. Most of the diseases are caused by fungi. In Bangladesh so far 17 chickpea diseases are recorded, 12 of which are caused by fungi[5]. Out of 12 fungal diseases Botrytis grey mould (BGM), wilt, root rot, blight and collar rot are the major ones[6].

Chickpea seeds in storage, carry a mycoflora of 'field' and 'storage' fungi. Field fungi gradually disappear and storage fungi then predominant. Most of the storage pathogen species are *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. The storage fungi may cause discoloration of the seeds and germination failure[7]. These fungi especially grow

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vigorously and initiate grain spoilage. They also bring about several undesirable changes making them unfit for consumption and sowing. Lot of research has been done in home and abroad on chickpea diseases and its control but information on storage mycoflora of chickpea seeds and its control are inadequate[8-10]. Keeping this in view the present research work was undertaken to find out the fungi associated with chickpea seeds in stored condition. This paper also deals with the management of storage mycoflora associated with the chickpea seeds.

Materials and Methods

The present study is carried on storage seeds of chickpea. Seed samples of BARI chola 1-9 were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. Samples were collected after harvesting and placed in clean brown paper bags, labeled properly and preserved at 4ºC in refrigerator for subsequent use.

The fungi were isolated from the samples following Tissue Planting Method on PDA medium[11] and Blotter method of ISTA. Two hundred seeds of each sample were placed on three layers of moist blotting paper (Whatman No. 1) in Petri plates. The seeds were washed with sterile water and then surface sterilized by dipping in 10% Chlorox solution for 5 minutes. Seeds were placed in each plate and incubated at 25 ± 2°C for 5 - 7 days.

Fungi grown in the seeds were transferred to separate PDA slants for further studies and preservation. Identification of the isolates were determined based on morphological characteristics observed under a compound microscope following standard literature[12-17]. Percentage of prevalence of fungi in different specimens was also recorded.

In vitro efficacy of fungicides on the radial growth of test fungi were evaluated. Five fungicides with different active ingradient(s), *viz*., Bavistin 50 WP, Tall 25 EC, Ridomil MZ Gold, Dithane M-45 and Greengel 72 WP were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. For each fungicide, a stock solution having the concentration of 10,000 ppm was prepared. The calculated amount of the stock solution of a fungicide was supplemented with sterilized PDA medium to get the final concentrations of 100, 200, 300, 400 and 500 ppm. Twenty ml of the supplemented medium of a particular concentration was poured in sterilized Petri plates and allowed to solidify. In the control set, required amount of sterile water instead of fungicide was added to the PDA medium. Then it was inoculated in the centre of the plate with a 5 mm of mycelial agar disc. Three replications were maintained in both the cases.

Efficacy of five plant extracts *viz*., *Azadirachta indica* A. Juss., *Allium sativum* L., *Citrus medica* L., *Datura metel* L. and *Psidium guajava* L. were evaluated against the same fungi. The desired parts of each plant were thoroughly washed in tap water, air dried and then used for preparation of extract. Leaf extracts were prepared by crushing known weight of fresh materials with ethanol in ratio of 1 : 1 (w/v). The mass of a plant part was squeezed

through fine cloth and the extracts were centrifuged at 3000 rpm for 20 minutes. The supernatants were filtered through Whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer conical flasks. The requisite amount of the filtrate was mixed with PDA medium in which plant extracts were in 5, 10 and 20% concentrations.

The radial growth of the colonies was measured at the 5th day of incubation. The per cent of growth inhibition of each test fungi was calculated by the formula described by Bashar and Rai[18].

Results and Discussion

A total of 9 species of fungi belongs to 6 genera and a sterile fungus were found to be associated with seeds of 9 varieties (BARI chola 1-9) of chickpea. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. niger* Van Tiegh., *A. fumigatus* Fresenius, *A. nidulans* Eidam, *Curvularia lunata* (Wakker) Boedijn, *Penicillium* Link, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers. The frequently associated fungi with seeds of chickpea were *A. alternata*, *A. flavus*, *A. fumigatus*, *C. lunata* and *Penicillium* sp. and these fungi were tried to control with selected fungicides and plant extracts.

Amongst the 5 fungicides used in the present investigation, Bavistin 50 WP, Dithane M-45 were systemic while Tall 25 EC was protective fungicides. Complete inhibition of the test pathogens were observed with Tall 25 EC at all the concentrations used and other fungicides inhibited the radial growth of the pathogen at different grades (Fig. 1 a-e).

Dithane M-45 and Tall 25 EC were responsible for complete inhibition of the radial growth of *A. alternata* at all the concentrations. Greengel 72 WP also showed 100% inhibition at 500 ppm whereas Bavistin and Ridomil showed only 73.33 and 66.67% inhibition at the same concentration. Bavistin, Greengel and Ridomil at 400 ppm concentration showed 66.67, 73.33 and 60.0% growth inhibition, respectively (Fig. 1a).

Amongst the 5 fungicides, Bavistin 50 WP, Tall 25 EC and Greengel 72 WP at different concentrations showed 100% inhibition of the radial growth of *A. flavus*. Ridomil MZ GOLD and Dithane M-45 showed 50% inhibition at 500 ppm and 42.86% inhibition at 400 ppm concentration (Fig. 1b).

Tall 25 EC and Bavistin 50 WP were completely inhibited the radial growth of *A. fumigatus* at all the concentrations used in the present study. Dithane M-45 also inhibited the radial growth completely at 500 ppm whereas Greengel 72 WP showed 54.55% and Ridomil MZ GOLD showed 60% inhibition (Fig. 1c).

The complete inhibition of the radial growth of *Curvularia lunata* was observed with Tall 25 EC at all the concentrations tested. Bavistin 50 WP and Greengel 72 WP also showed 100% inhibition of growth at 400 and 500 ppm concentrations whereas the per

cent inhibition of Dithane M-45 and Ridomil MZ Gold was not so good. They showed 76.66 and 74.78% inhibition at 500 ppm concentration, respectively (Fig. 1d).

Growth of the *Penicillium* sp. was completely inhibited with Tall 25 EC at all concentrations used. Bavistin 50 WP and Greengel 72 WP showed 100% inhibition at 500 ppm and 70% inhibition at 400 ppm concentration. Dithane and Ridomil inhibited 75.0 and 83.34% growth of the fungus at 500 ppm concentration and 64.29 and 42.86% inhibition at 400 ppm concentration, respectively (Fig. 1e).

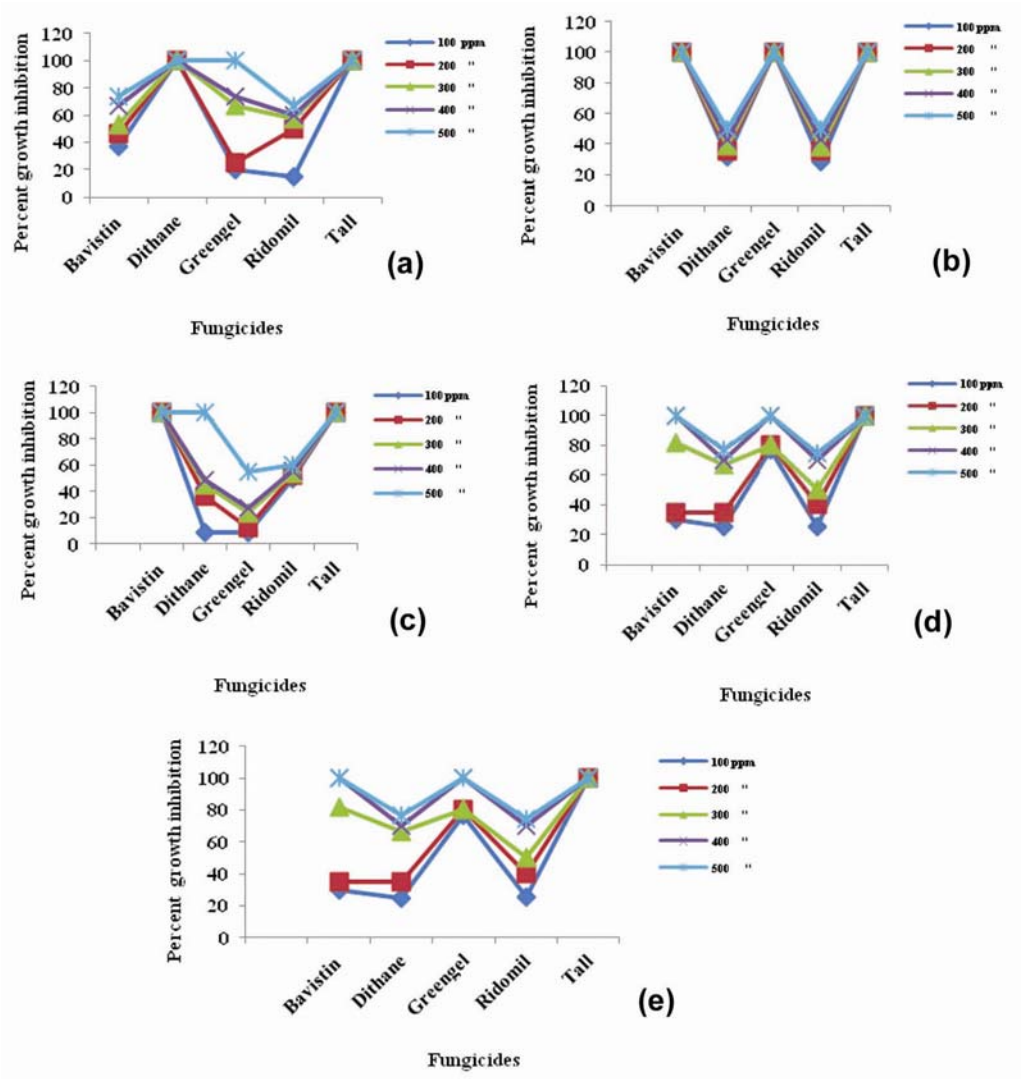

Fig. 1. Per cent inhibition of radial growth of a. *Alternaria alternata*, b. *Aspergillus flavus*, c. *A. fumigatus*, d. *Curvularia lunata* and e. *Penicillium* sp. owing to fungicides at different concentrations.

Amongst the five fungicides, Tall 25 EC showed best result and Bavistin 50 WP showed least percentage of inhibition.

Antifungal properties of ethanol extracts of five plant parts *viz*., *Azadirachta indica, Allium sativum*, *Citrus medica*, *Datura metel* and *Psidium guajava* at 5, 10 and 20% concentrations were evaluated on the test fungi. *Allium sativum* was found most efficient inhibitor of the test fungi followed by *D. metel*, *A. indica, C. medica* and *P. guajava* (Fig. 2 a-e).

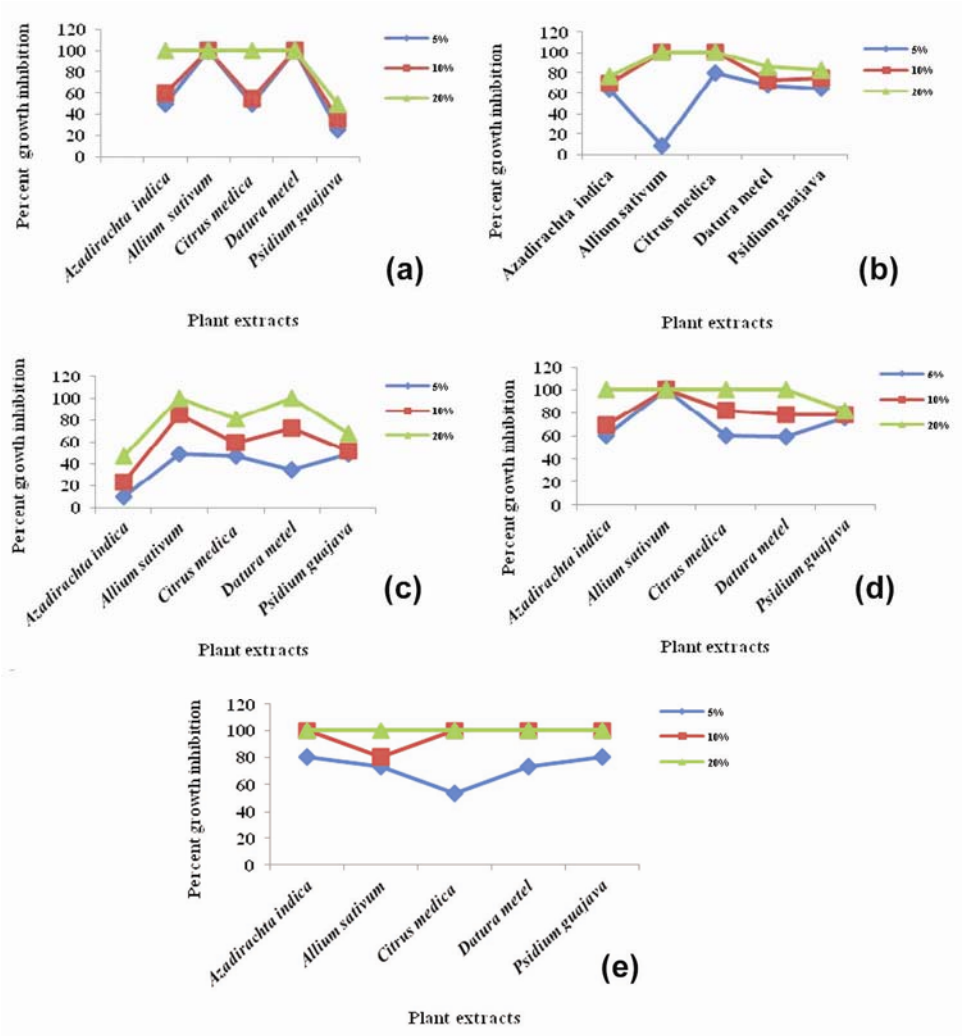

Fig. 2. Per cent inhibition of radial growth of a. *Alternaria alternata*, b. *Aspergillus flavus*, c. *A. fumigatus*, d. *Curvularia lunata* and e. *Penicillium* sp. owing to plant extracts at different concentrations.

Amongst 5 plant extracts used in this experiment ethanol extract of *Datura metel* and *Allium sativum* showed complete inhibition of radial growth of *Alternaria alternata* at all the concentrations. *Citrus medica* and *Azadirachta indica* also showed 100% inhibition at 20% concentration whereas *Psidium guajava* showed 50% inhibition at the same concentration. *C. medica, A. indica* and *P. guajava* showed 55.0, 60.0 and 35.0% growth inhibition, respectively at 10% concentration. (Fig. 2a).

Ten and 20 per cent ethanol extracts of *A. sativum* and *C. medica* were responsible for complete inhibition of radial growth of *A. flavus* whereas the 20% concentration of *A. indica, D. metel* and *P. guajava* showed 77.05, 86.0 and 83.5% inhibition of the fungal growth, respectively. These 3 ethanol plant extracts showed 70.0, 72.5 and 75.0% inhibition at 10% concentration (Fig. 2b).

Twenty per cent ethanol extracts of *Allium sativum* and *Datura metel* were responsible for 100% inhibition of the radial growth of *A. fumigatus* whereas *A. indica, C. medica* and *P. guajava* showed 46.67, 81.25 and 67.74% inhibition of the fungus at the same concentration. The ethanol extracts of *A. indica, A. sativum, C. medica, Datura metel* and *P. guajava* showed 23.33, 84.62, 59.36, 73.08 and 51.62% inhibition at 10% concentration, respectively (Fig. 2c).

Ethanol extract of *A. sativum* showed 100% inhibition of radial growth of *Curvularia lunata* at all the concentrations used. Twenty percent concentration of *A. indica, C. medica* and *D. metel* also showed complete inhibition of *Curvularia lunata* whereas *P. guajava* showed 81.82% inhibition at the same concentration. The extracts of *A. indica, C. medica, D. metel* and *P. guajava* showed 69.70, 81, 82, 78.13 and 78.79% inhibition of the growth at 10% concentration, respectively (Fig. 2d).

Twenty per cent ethanol extracts of *A. indica, A. sativum, C. medica, D.metel* and *P. guajava* were responsible for complete inhibition of radial growth of *Penicillium* sp. 10% ethanol extract of these 5 plants also showed 100% inhibition except *A. sativum* which showed 80.34% inhibition (Fig. 2e).

The extract of different parts of higher plants have been reported to exhibit antifungal properties under laboratory trials[19]. Plant parts and their constituents of some higher plants have already been reported to be of successful nature of fungitoxicant because of their lesser phytotoxicity, systemicity, easily vertigratability and favourable effect for the growth of host.

Bashar and Rai[18] have reported that lime fruit peel essential oil components inhibit linear growth on spore germination of *P. italicum*, *P. digitatum* and *Geotrichum canium.*

Mohana *et al*.[19] from India reported that methanol extracts of *Acacia nilotica, Caesalpinia coriaria, Decalepis hamiltonii, Emblica officinalis* and *Mimosops elengi* showed significant antifungal activity at 3500 µg/ml concentration on seed pathogens *viz., Alternaria alternata, Aspergillus flavus, Curvularia lunata, Drechslera oryzae, D. halodes,*

Fusarium moniliforme, Pyricularia oryzae and *Trichoconis padwickii* by poisoned food technique.

Chowdhury *et al*.[20] reported that the presence of 5 pathogenic fungi *viz*., *Alternaria alternata, Curvularia lunata, Drechslera oryzae, Fusarium moniliforme* and *Pestalotiopsis guepinii* associated with rice grains were completely controlled *in vitro* at different concentrations of Tall 25 EC. Antifungal activity of ethanol extracts of *Tagetes erecta, Datura metel, Senna alata, Azadirachta indica, Citrus medica, Mangifera indica, Artocarpus heterophyllus, Asparagus racemosus, Nerium indicum* and *Allium sativum* completely inhibited the radial growth of the test fungi at 20% concentration.

Singh and Singh[9] treated the seeds of Broad bean (*Vigna faba*) with Dithane M-45, Ceresan, Bavistin and Vitavax. Among the treatments seed borne mycoflora was most effectively controlled by Dithane M- 45.

Salam[10] worked with storage chickpea seeds collected from two different locations. *Aspergillus flavus, A. niger, Fusarium oxysporum, F. Moniliforme, Penicillium notatum, Alternaria alternata, Sclerotium rolfsii* and *Botrytis cinera* were found to be associated with the stored seeds. He tested the efficacy of Vitavex- 200 and hot water treatment on the fungi associated with chickpea seeds and found seed treatment with Vitavex-200 at the rate of 2.5 g/kg and 56ºC hot water showed the most effective results.

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