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IN-VITRO STUDIES OF SOME SELECTED BOTANICALS AND BAU-BIOFUNGICIDE ON MYCELIAL GROWTH AND CONIDIAL GERMINATION OF *Cercospora arachidicola* AND *Cercosporidium personatum*

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Abstract

Efforts have been made to assess some plant extracts namely, *Lycopersicon esculentum*, *Tagetus patula*, *Achras sapota*, *Azadirachta indica*, *Datura metel*, *Cymbopogon citratus*, *Polyalthia longifolia*, *Allium sativum* and *Allium cepa* *in vitro* for the management of leaf spot (tikka) disease of groundnut cultivar Dhaka-1 caused by *Cercospora arachidicola* and *Cercosporidium personatum*. Results indicated that all the tested plant extracts and BAU-Biofungicide suppressed the growth of mycelium and inhibition of conidial germination of *C. arachidicola* and *C. personatum*. Among the treatments, the leaf extracts of *L. esculentum* showed the most effective followed by leaf extract of *D. metel*, *A. indica* and BAU-Biofungicide in case of mycelial growth and conidial germination. Other plant extracts also had inhibitory effects. In case of conidial germination and germination inhibition, the least effective plant extract was *C. citratus*. Leaf extract of *A. sapota* was the least effective in case of mycelial growth.

Keywords: *In-vitro*, Botanicals, BAU-Biofungicide, Mycelial growth and Conidia germination

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important annual legume crop belonging to the family Fabaceae growing in many tropical and subtropical countries of the world (Wudiri and Fatoba, 1992). It is a multipurpose and highly nutritious crop containing oil, food and its foliage or haulm provides a valuable fodder for livestock. More than 40 fungal diseases attack groundnut (Jackson and Bell, 1969), but in Bangladesh, the crop is subjected to attack by twenty one diseases (Talukder, 1974; Ahmed and Hossain, 1985). Among the diseases, early leaf spot caused by *Cercospora arachidicola* and late leaf spot caused by *Cercosporidium personatum* are the most devastating and economically important foliar fungal disease and major yield reducing factor of groundnut worldwide (Backman and Crawford, 1984; Khaleque, 1985). The early and late leaf spots of groundnut although caused by two fungal species, *C. arachidicola* and *C. personatum*, they are commonly referred together as Tikka disease. This disease results in early defoliation thereby affecting the pod formation (Worthington and Smith, 1973). Loss in pod yield due to the diseases was recorded as 70% in groundnut (Subrahmanyam *et al.*, 1980). The yield loss was calculated in the groundnut variety Dhaka-1 due to early and late leaf spot (Tikka) by over 30-48%

in Bangladesh (Hossain *et al.*, 2005). In addition to direct yield loss, they hamper seed quality by reducing seed size and seed weight (Souta, 1912; Arthur, 1929) and oil content (Gupta *et al.*, 1988). Severe attack of the disease resulting in heavy defoliations of groundnut leaves (Harrison, 1969). The disease can be controlled by developing resistant varieties, seed treatment with non-conventional chemicals (Maiti *et al.*, 2005), spraying fungicides, influence by sowing times (Naidu and Vasanthi, 1995), by using indigenous medicinal plants and biological control means (Kishore and Pande, 2005). The most acceptable method for controlling this disease is cultivation of resistant variety but there was no absolute resistant variety in the world (Wynne *et al.*, 1991). Increasing concerns about environment hazards caused by excessive usage of chemical fungicide, necessitates the development of more economical and eco-friendly alternative components of disease management. Biological control represents a less cost, environmental friendly natural and ecological approach for controlling diseases that reduces chemical inputs and their effect on environment. The main purpose of the study was to evaluate selected nine plant extracts for their *in vitro* antifungal activity against *C. arachidicola* and *C. personatum*.

Materials and Methods

Laboratory experiment was carried out at Disease Resistant Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh following Completely Randomized Design (CRD) with four replications for bioassay of nine botanical extracts, BAU-Biofungicide (*Trichoderma* based preparation) and Bavistin (Carbendazim) against mycelial growth of *C. arachidicola* and *C. personatum*. The treatments were also tested in the present experiment following cavity slide technique to find out their effect on conidial germination of *C. arachidicola* and *C. personatum*.

Preparation of plant extracts

Plant extracts were prepared from fresh leaves of tomato (*Lycopersicon esculentum*), marigold (*Tagetes patula*), sapota (*Achras sapota*), neem (*Azadirachta indica*), datura (black) (*Datura metel*), lemongrass (*Cymbopogon citratus*) and debdar (*Polyalthia longifolia*) and bulb of garlic (*Allium sativum*) and onion (*Allium cepa*). The collected fresh leaf samples of different plants were washed in running tap water to make free from dust. The plant materials were cut into small pieces and extracts were prepared by grinding in a mortar and pestle followed by crashing plant parts in an electric blender with sterilized distilled water at different doses. The mixtures were filtered through linoleum cloth. The extracts were kept in conical flasks separately before use. Concentration (weight/volume) of tomato leaf, datura leaf (black) and sapota leaf was 20%, neem leaf, marigold leaf, garlic clove and onion bulb was 25%. Lemongrass, BAU-Biofungicide and Bavistin were used tested at 1:2, 2.5% and 0.1% concentration, respectively.

Bioassay of botanicals, BAU-Biofungicide and Bavistin on *C. arachidicola* and *C. personatum*

Carrot leaf extract agar medium was prepared and poured into 90 mm Petri plates at 20 ml/plate. After solidification, three 5 mm discs of the medium were scooped from three places maintaining equal distance of 4 cm from the centre using a sterilized disc cutter. One millimeter of each of plant extracts and suspensions of BAU-Biofungicide and Bavistin was put into each hole and the plates were stored overnight in refrigerator for diffusion of the test materials into the medium surrounding the hole. Next day, the plates were inoculated at the center with 6 mm blocks of 15 days old culture of *C. arachidicola* and *C. personatum*. Three plates (replications) were maintained for each material. Control plates did not receive any material. To prevent contamination, the plates were covered with the Para film and the plates were incubated at 24 ± 1°C.

The isolates of *C. arachidicola* and *C. personatum* were grown on carrot leaf extract in Petri plates for 12 days at room temperature (24 ± 1°C). The culture plates were kept under NUV light for 3 days for maximum production of conidia. Conidia were collected from the plates by scraping with a sterilized glass slide and conidial suspension was prepared in sterilized distilled water. The suspension was filtered through two-ply cheese cloth to remove mycelial fragments and lumps of agar. The concentration of conidia in suspension was adjusted to 2x10⁴ per milliliter using a hemocytometer (Krishna *et al.*, 2001).

Observation was made regularly to record the mycelial growth. After inoculation periods of 10, 13 and 15 days the linear growth of mycelial of *C. arachidicola* and *C. personatum* was measured (McKeen *et al.*, 1986; Nene and Thapliyal, 1993; Islam *et al.*, 2001) and percent inhibition of growth was calculated using the following formula as suggested by Sundar *et al.* (1995):

$$\text{Inhibition (\%)} = \frac{X-Y}{X} \times 100$$

Where,

X = Mean mycelial growth (radial) of pathogen in control plate

Y = Mean mycelial growth (radial) of pathogen in treatment

Growth inhibition of *C. arachidicola* and *C. personatum*.

To assay the antifungal activity of the botanical extracts, Bavistin and BAU-Biofungicide, 50µl of conidial suspension and equal volume of the suspension of test materials transferred to the well of each cavity slide and mixed thoroughly. Three single cavity slides (replications) were used for each treatment. All slides were kept in a humid chamber prepared by lining 90 mm diameter Petri dishes with wet tissue paper and incubated in the dark at 24± 1°C. The slides were directly observed under light microscope for conidial germination at 24, 48 and 72 hours after incubation. Immediately after inoculation, a drop of lacto phenol-cotton blue was added to each well to prevent further germination of conidia. Fifty conidia in a well were observed under a compound microscope and number of germinated and non-germinated conidia was counted. Percentage inhibition of conidial germination in each treatment was calculated from the formula:

Percentage inhibition = (number of conidia germination in control–number of conidia germination in treatment/ number of conidia germination in control × 100 (Krishna *et al.*, 2001).

Results and Discussion

Effect of nine different selected botanicals, Bavistin and BAU-Biofungicide on mycelial growth of *C. arachidicola* and *C. personatum*

The efficacy of different nine selected botanicals, Bavistin and BAU-Biofungicide were evaluated against mean mycelial growth and growth inhibition of *C. arachidicola* and *C. personatum* at 10, 13, 15 days after inoculation (DAI). The results are presented in Table 1.

Table 1. *In-vitro* evaluation of nine different selected botanicals, Bavistin and BAU-Biofungicide on mycelial growth of *C. arachidicola* and *C. personatum*

| Treatments | 10 DAI | | 13 DAI | | 15 DAI | |
|--------------------------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|
| | Mycelium growth (cm) | Growth inhibition (%) | Mycelium growth (cm) | Growth inhibition (%) | Mycelium growth (cm) | Growth inhibition (%) |
| <i>Tagetus patula</i> | 3.9 | 41.9 | 4.5 | 50.0 | 4.7 | 47.8 |
| <i>Datura metel</i> | 1.3 | 80.8 | 1.5 | 83.3 | 1.6 | 81.9 |
| <i>Azadirachta indica</i> | 2.9 | 57.9 | 2.2 | 75.6 | 2.3 | 74.4 |
| <i>Lycopersicon esculentum</i> | 0.9 | 86.3 | 1.1 | 87.4 | 1.2 | 86.7 |
| <i>Allium sativum</i> | 4.2 | 38.0 | 4.6 | 49.3 | 4.6 | 48.6 |
| <i>Achras sapota</i> | 5.6 | 17.3 | 6.4 | 28.6 | 8.9 | 14.4 |
| <i>Polyalthia longifolia</i> | 2.5 | 62.6 | 3.9 | 56.7 | 4.4 | 51.4 |
| <i>Cymbopogon citratus</i> | 5.2 | 23.5 | 8.5 | 5.6 | 8.9 | 0.8 |
| <i>Allium cepa</i> | 5.3 | 21.3 | 5.9 | 34.1 | 6.0 | 33.8 |
| BAU-Biofungicide | 1.9 | 71.8 | 2.2 | 75.9 | 2.4 | 73.7 |
| Bavistin | 1.0 | 85.2 | 1.4 | 84.8 | 1.4 | 84.8 |
| Control (untreated) | 6.8 | - | 9.0 | - | 9.0 | - |
| LSD(P \geq 0.001) | 1.04 | - | 3.24 | - | 3.42 | - |

Data represent the means of three replications

All treatments with botanicals, Bavistin and BAU-Biofungicide reduced mycelial growth on culture plates significantly over control. At 10 days after inoculation (DAI), the mean mycelial growth varied from 0.9 to 6.8 cm, where maximum and minimum mycelial growths were recorded under control (plain water) and leaf extract of *Lycopersicon esculentum*, respectively. Radial colony diameter under Bavistin, leaf extracts of *Datura metel* and BAU-Biofungicide was statistically similar to that under leaf extract of *Lycopersicon esculentum*. Maximum of 86.3% growth inhibition over control was obtained with leaf extract of *Lycopersicon esculentum* followed by Bavistin, *Datura metel*, BAU-Biofungicide, leaf extract of *Polyalthia longifolia* and *Azadirachta indica*. Minimum of 17.3% growth inhibition was recorded under the treatment with leaf extract of *Achras sapota* leaf. At 13 and 15 DAI, trends in mean mycelial growth and growth inhibition were identical as recorded at 10 DAI (Table 1). The findings of this trial are in agreement with the results of Natarajan *et al.* (2005), Kishore and Pande (2005), Patni *et al.* (2005), Buckingham (1993), Srivastava and Lal (1997).

Effect of nine different selected botanicals, Bavistin and BAU-Biofungicide on conidial germination of *C. arachidicola* and *C. personatum*

The comparative effects of different nine selected botanicals, Bavistin and BAU-Biofungicide on conidial germination of *C. arachidicola* and *C. personatum* are presented in Table 2. All the botanicals, Bavistin and BAU-Biofungicide significantly reduce germination of conidia of the pathogens in the well of cavity slides compared to control (only water) at 24, 48 and 72 hours after incubation (Table 2).

At 24 hours of incubation, conidial germination ranged 0.0 to 68.0%, where the lowest conidial germination was observed in leaf extract of *Lycopersicon esculentum*, which was followed by leaf extract of *Datura metel*, Bavistin and leaf extract of *Azadirachta indica*. The highest conidial germination was recorded in untreated control. The extracts of onion bulb, *Tagetus patula*, *Achras sapota* and *Cymbopogon citratus* showed statistically similar effect on conidial germination after 24 hours of incubation. Among the treatments, leaf extract of *Lycopersicon esculentum* showed maximum (100%) inhibition of conidial germination. All the botanical extracts and BAU-Biofungicide inhibited conidial germination over 80% except leaf extract of *Tagetus patula*, *Cymbopogon citratus* and bulb of *Allium cepa*. BAU-Biofungicide, Bavistin, leaf extract of *Datura metel* and *Azadirachta indica* showed statistically similar effect on the inhibition of conidial germination (Table 2).

Table 2. Effect of nine different selected botanicals, Bavistin and BAU-Biofungicide on conidial germination of *C. arachidicola* and *C. personatum* following cavity slide method

| Treatments | 24 hour | | 48 hour | | 72 hour | |
|--------------------------------|--------------------------|----------------------------|--------------------------|----------------------------|--------------------------|----------------------------|
| | Conidial germination (%) | Germination inhibition (%) | Conidial germination (%) | Germination inhibition (%) | Conidial germination (%) | Germination inhibition (%) |
| <i>Tagetus patula</i> | 30.7 | 69.3 | 46.7 | 53.3 | 52.0 | 48.0 |
| <i>Datura metel</i> | 1.3 | 98.7 | 16.0 | 84.0 | 25.3 | 74.7 |
| <i>Azadirachta indica</i> | 9.3 | 90.7 | 24.0 | 76.0 | 29.3 | 70.7 |
| <i>Lycopersicon esculentum</i> | 0.0 | 100.0 | 1.3 | 98.7 | 8.0 | 92.0 |
| <i>Allium sativum</i> | 18.7 | 81.3 | 26.7 | 73.3 | 39.3 | 60.7 |
| <i>Achras sapota</i> | 29.3 | 70.7 | 25.3 | 74.7 | 36.0 | 64.0 |
| <i>Polyalthia longifolia</i> | 21.3 | 78.7 | 30.7 | 69.3 | 26.7 | 73.3 |
| <i>Cymbopogon citratus</i> | 36.0 | 64.0 | 56.0 | 44.0 | 57.3 | 42.7 |
| <i>Allium cepa</i> | 32.0 | 68.0 | 36.0 | 64.0 | 37.3 | 62.7 |
| BAU-Biofungicide | 14.7 | 85.3 | 28.0 | 72.0 | 26.7 | 73.3 |
| Bavistin | 9.3 | 90.7 | 16.0 | 84.0 | 17.3 | 82.7 |
| Control (untreated) | 68.0 | 32.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| LSD(P ≥ 0.001) | 20.54 | 20.54 | 22.17 | 22.17 | 21.37 | 21.37 |

Data represent the means of three replications

At 48 hours of incubation, significantly the highest conidial germination (100%) was recorded from control, whereas the lowest germination was found in leaf extract of *Lycopersicon esculentum* followed by *Datura metel* and Bavistin. The germination of conidia was 24% in leaf extract of *Azadirachta indica*. Conidial germination in the suspension of BAU-Biofungicide, extracts of *Allium sativum*, *Polyalthia longifolia*, *Achras sapota* and *Allium cepa* was statistically similar. In case of inhibition of conidial germination, the highest per cent of inhibition was observed in leaf extract of *Lycopersicon esculentum*, whereas the lowest was found in plain water (control).

At 72 hours of incubation, conidial germination was reduced significantly over control under all the treatments with plant extracts, Bavistin and BAU-Biofungicide. Conidial germination ranged 8.0 to 100.0%, while the lowest conidial germination was recorded in leaf extract of *Lycopersicon esculentum* followed by Bavistin, leaf extracts of *Datura metel*, BAU-Biofungicide, leaf extracts of *Azadirachta indica* and *Polyalthia longifolia*. The highest (100.0%) conidial germination was observed in plain water (control). The conidial germination inhibition ranged 0.0 to 92.0%, where maximum and minimum conidial germination inhibition was recorded from the wells containing leaf extract of *Lycopersicon esculentum* and water (control), respectively (Table 2). The findings of the present research work are in consonance with the findings of Natarajan *et al.* (2005), Kishore and Pande (2005), Abdulrahman and Alkhail (2005), Aage *et al.* (2003).

Among the treatments, the most effective was the leaf extracts of *Lycopersicon esculentum* followed by *Datura metel*, BAU-Biofungicide, leaf extract

of *Azadirachta indica* in case of mycelial growth and conidial germination. Other plant extracts also had inhibitory effects but not as much as the leaf extracts of *Lycopersicon esculentum*, *Datura metel*, BAU-Biofungicide, leaf extract of *Azadirachta indica*. Leaf extract of *Achras sapota* was the least effective against mycelial growth of *C. arachidicola* and *C. personatum*. In case of conidial germination and germination inhibition the least effective plant extract was *Cymbopogon citratus*. The potentials of these plant extracts for pathogen control have not been fully realized largely because the experiment was performed *in vitro*. However, their effectiveness in field condition could be of a potential advantage as it will help to determine the *in vivo* inhibitory effect of the botanicals and BAU-Biofungicide.

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