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IN VITRO EFFECTS OF PLANT EXTRACTS AND FUNGICIDES TO CONTROL WILT OF BRINJAL (*SOLANUM MELONGENA* L.)

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Abstract

Five fungicides *viz*., Bavistin DF, Capvit 50 WP, Dithane M-45, Greengel 72 WP and Tilt 250 EC were selected to evaluate *in vitro* efficacy against *Fusarium oxysporum* and *F. solani* at 100, 200, 300, 400 and 500 ppm following poisoned food technique. Bavistin, Capvit and Tilt were found to be most effective inhibitor against the test pathogens isolated from brinjal wilt. Bavistin and Tilt completely inhibited the growth of *F. oxysporum* and *F. solani* at all the tested concentrations. Out of five plant extracts, *Allium sativum* identified as the promising botanical fungicide for testing against wilted plants of brinjal. The result of present study will be helpful in suggesting some effective plant extracts and fungicides for controlling wilting of brinjal.

Introduction

Brinjal (*Solanum melongena* L.) is one of the most popular vegetables in Bangladesh belongs to Solanaceae[1]. It is popularly known as eggplant, aubergine, melongene, brinjal, or guinea squash. Eggplant is the second most important vegetable crop next to potato in Bangladesh in respect of its acreage and production[2]. In Bangladesh, brinjal is cultivated widely in the field for commercial purpose as well as in the homeyard and kitchen garden. The total area used for kharif and rabi brinjal cultivation in Bangladesh during 2013 - 2014 growing year was 42314 and 73409 acres with a total annual production of 133633 and 310510 metric tons, respectively[3]. The yield potential of eggplant is low in Bangladesh compared to other countries. Among the various constraints for brinjal cultivation, diseases play an important role. The crop is known to suffer from more than 70 different diseases[4]. About 13 different diseases so far recorded in Bangladesh[5,6]. Major fungal diseases of brinjal are leaf spot, fruit rot, black rot, leaf blight, foot rot, wilt, etc. Among those diseases Fusarium wilt of eggplant has been treated as one of the major constrains in eggplant cultivation in Bangladesh[7].

Nowadays, many inorganic and organic fungicides are used frequently to control plant diseases[8]. Various workers in different countries evaluated the efficacy of various fungicides against *Fusarium oxysporum* and *F. solani* under laboratory and field conditions[9-11]. All these fungicides are not available in Bangladesh. Therefore, it is necessary to test the efficacy of the fungicides against the pathogens.

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In recent years, some workers on the fungi toxicity of extracts of various parts of higher plants have indicated the possibility of their exploitation as natural fungi toxicants for controlling plant diseases[12-15]. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides[16]. A few attempts have been made for the control of mycoflora associated with brinjal by fungicides and plant extracts. Therefore, the present investigation was ascertained to investigate the inhibitory effect of extracts of five plant species and fungi toxicity of five fungicides on isolated pathogen of wilted brinjal under *in vitro* conditions.

Materials and Methods

Wilted brinjal plants were collected from selected fields of Bangladesh Agriculture Development Corporation (BADC) in Gazipur and research field of the Botanical Garden at Curzon Hall, Dhaka University during October, 2015 to April, 2016. Samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolation of fungi following "Tissue planting method" on PDA medium. Fifty inocula, each measuring 2 mm^2 sized were cut separately with a pair of sterilize scissors and kept in a separate sterilized Petri plate. The inocula were washed with sterile water and then surface sterilized by dipping in 10% Chlorox solution for three minutes. The inocula were again washed with sterile water. A total of 30 inocula were placed separately on 10 sterilized Petri plates containing 15 ml of PDA medium with an addition of 1 drop (ca. 0.03 ml) of lactic acid and incubated in an incubator (25 ± 2ºC) for 7 days. Identification of the isolates was made following the standard reference books[17,18].

Five fungicides *viz*., Bavistin DF, Capvit 50 WP, Dithane M-45, Greengel 72 WP and Tilt 250 EC were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. For each fungicide, a stock solution of 10000 ppm was prepared. The calculated amount of stock solution of fungicide as supplemented with sterilized PDA medium to get the concentration of 100, 200, 300, 400 and 500 ppm, respectively. In control set, required amount of water was used instead of a fungicide. Then 15 ml of medium was poured in each Petri plate and allowed them to solidify.

For *in vitro* effect of five plant extracts, *viz*., *Allium sativum* (bulb), *Azadirachta indica* (leaf), *Cassia alatam* (leaf), *Citrus limon* (leaf) and *Datura metel* (leaf) were selected on the radial growth of test pathogens. The desired parts of each plant were thoroughly washed in tap water, air dried and were prepared by crushing to 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 particles. The supernatants were filtered through Whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer flask. The requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10, 15

and 20% concentration. In control set, required amount of water was used instead of plant extract. Five mm mycelial agar disc cut from the margin of actively growing culture of test pathogens and then it was inoculated at the centre of the plate. All the Petri plates were incubated at 25 ± 2°C. The radial growth of the test pathogen colonies were measured after 7 days. The per cent growth inhibition of each test pathogen was calculated using the following formula:

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

where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment. The results were statistically analyzed by t test following Steel and Torrie[19].

Results and Discussion

Five fungi *viz., Aspergillus flavus, A. fumigatus, A. niger*, *Fusarium oxysporium* and *F. solani* were isolated from the wilted brinjal. Identification of these fungi was determined following the standard reference books[17,18]. Among the isolated fungi *Fusarium oxysporium* and *F. solani* were selected as test pathogens owing to their higher percentage of occurrence and previous reports as pathogenic organisms[20].

The results of fungicides on the radial growth of *Fusarium oxysporum* and *F. solani* at 100, 200, 300, 400 and 500 ppm are presented in Tables 1 and 2. Out of five fungicides, complete inhibition of the radial growth of *Fusarium oxysporum* was observed with Bavistin and Tilt at all the tested concentrations (Table 1). Greengel, Dithane and Capvit showed 73.80, 58.33 and 30% growth inhibition of *Fusarium oxysporum*, respectively at 500 ppm (Table 1). The complete inhibition of growth of *Fusarium solani* was also observed with Bavistin and Tilt at all the concentrations (Table 2). Greengel and Dithane showed 68.28 and 48.57% growth inhibition of *Fusarium solani*, respectively at 500 ppm (Table 2). Bashar *et al.*[14] reported the effects of Bavistin, Dithane and Tall on the radial growth of *Fusarium oxysporum* and *F. solani* at 400 ppm only. Chakraborty *et al.*[11] reported that, at 0.5% dose, bavistin happened to be the most efficient one contributing the highest inhibition (83.7%) of growth of the *F. solani* causing wilt of brinjal under *in vitro* condition. Bashar[21] reported that bavistin checked the complete growth of *F. oxysporum* f. sp. *ciceri,* causal agent of chickpea wilt at 100 ppm concentration. He also noted that dithane failed to check the growth of the pathogen completely even at 3,000 ppm.

Laboratory evaluation of fungicides revealed that all the fungicides cause partial or complete inhibition of *F. oxysporum* and *F. solani* at 500 ppm (Tables 1 and 2). The same fungicides also showed different effects on different pathogens in the present investigation due to the selection of different strains of test pathogens.

Results of plant extracts on the radial growth of *F. oxysporum* and *F. solani* at 5, 10, 15 and 20% concentration are shown in Tables 3 and 4. All the plant extracts showed varied degree of growth inhibition of the pathogens at different concentrations. Out of the five plant extracts, *Allium sativum* and *Cassia alata* showed 100% radial growth inhibition of *F. oxysporum* at 20% concentration followed by *Azadirachta indica* (55.55%), *Datura metel*

Table 1. Per cent inhibition of radial growth of *Fusarium oxysporum* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Bavistin DF	100[a]	100[a]	100[a]	100[a]	100[a]
Capvit 50 WP	10.00	15.00	20.00[c]	25.00[c]	30.00[c]
Dithane M-45	22.22[b]	30.55[b]	52.77[b]	55.55[b]	58.33[b]
Greengel 72 WP	38.09[b]	47.61[b]	59.52[b]	64.28[a]	73.80[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]

a, b and c indicate significance of t value at p = 0.001, 0.01 and 0.05, respectively. In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT).

Table 2. Per cent inhibition of radial growth of *Fusarium solani* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Bavistin DF	100[a]	100[a]	100[a]	100[a]	100[a]
Capvit 50 WP	17.14[c]	28.57[b]	35.71[b]	100[a]	100[a]
Dithane M-45	28.57[b]	35.71[b]	44.28[a]	45.71[a]	54.47[a]
Greengel 72 WP	14.63[c]	24.39[b]	48.78[b]	56.09[b]	68.29[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]

(33.33) and *Citrus limon* (25%). The inhibition of the test pathogens increases with the increase of the concentration of the plant extracts in culture medium (Table 3). The order of effectiveness against *F. oxysporum*at 20% was *A. sativum* = *C. alata* (100%) > *A. indica* (55.55%) > *D. metel* (33.33%) > *C. limon* (25%) (Table 3). The highest inhibition of radial growth of *F. solani* was observed with *A. sativum* and *C. alata* at 20% which was followed by *A. indica* (80.48%), *C. limon* (60.97%) and *D. metel* (28.12%) (Table 4). The order of effectiveness against *F. solani*at 20% was *A. sativum* = *C. alata* (100%) > *A. indica* (80.48%) > *C. limon* (60.97%) > *D. metel* (28.12%) (Table 4).

Bashar and Chakma[13] reported that the plant extracts of *C. alata*, *A. indica*, *A. sativum* and *D. metel* showed 74.78, 62.03, 34.21 and 34.18% growth inhibition of *F. oxysporum* at

20% concentration, respectively. Again, *D. metel*, *A. indica*, *A. sativum* and *C. alata* showed 66.67, 57.14, 53.85 and 50% growth inhibition of *F. solani* at 20% concentration, respectively. The same plant extracts also showed different effects on same pathogens in the present investigation. This variation might be due to selection of different strains of test pathogens.

Table 3. Effect of plant extracts on the radial growth of *Fusarium oxysporum* at different concentrations.

Name of plants	% inhibition of radial growth of the pathogen at different concentrations (%)			
	5	10	15	20
Allium sativum	100[a]	100[a]	100[a]	100[a]
Azadirachta indica	38.88[b]	44.44[a]	48.88[a]	55.55[b]
Cassia alata	49.14[b]	58.25[b]	76.07[b]	100[a]
Citrus limon	10.00[c]	15.00[c]	20.00[c]	25.00[c]
Datura metel	15.15[c]	24.24[b]	30.30[b]	33.33[b]

Table 4. Effects of plant extracts on the radial growth of *Fusarium solani* at different concentrations.

Name of plants	% inhibition of radial growth of the pathogen at different concentrations (%)			
	5	10	15	20
Allium sativum	55.55[b]	66.66[b]	100[a]	100[a]
Azadirachta indica	58.53[a]	65.85[a]	75.60[a]	80.48[a]
Cassia alata	53.33[b]	64.44[a]	86.66[a]	100[a]
Citrus limon	36.58[b]	43.90[b]	51.21[b]	60.97[a]
Datura metel	12.50[c]	18.75[c]	21.87[c]	28.12[b]

The results of this study identified Bavistin, Capvit and Tilt for wilted plants of brinjal as best inhibiting chemical fungicides. Out of five plant extracts, *A. sativum* identified as promising botanical fungicide for further investigation against wilted plants of brinjal.

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