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Screening of Different Eggplant Cultivars against Wilt Disease Caused by Fungi, Bacteria and Nematodes

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ABSTRACT: An experiment was conducted in the Field of Sher-e-Bangla Agricultural University, Dhaka to screen out the resistant cultivars of eggplant against wilt disease. Eight cultivars viz. Nayantara, Singhnath, Dhundul, Kazla, Marich Begun Luffa, Kata Begun and Uttara were used as treatments. At 55 days after transplanting (DAT) the cultivar Luffa exhibit the highest bacterial wilt incidence (80%) and the lowest wilt incidence was recorded in the cultivar Kata Begun (30%). At 90 DAT the highest Fusarium and Nemic wilt incidence was recorded in the cultivar Luffa and the lowest wilt incidences were recorded in the cultivar Kata Begun. The highest shoot height was recorded in the cultivar Kata Begun and the lowest shoot height was recorded in the cultivar Singhnath. The highest gall number was recorded in the cultivar Luffa and the lowest gall number was recorded in the cultivar Kata Begun. The highest yield per hectare (29.84 t/ha) was recorded in the cultivar Nayantara and the lowest yield (10.50 t/ha) was recorded in the cultivar Dhundhul. Among the cultivars Kata Begun graded as resistant for both Bacterial, Fungal and Nemic wilt.

Key words: Disease incidence, Screening, Fusarium, Wilting, Nematode

Introduction

Eggplant or Brinjal (*Solanum melongena* L.) belongs to the family Solanaceae is the most important and widely consumed nutritious vegetable in Bangladesh. Eggplant is the second most important vegetable crop next to potato in Bangladesh in respect of acreage and production (BBS, 2005). It is cultivated a commercially throughout the tropical and subtropical region of the world. It is also popular in other countries like Balkan area, France, Indonesia, Italy, Japan, Mediterranean, Turkey and United states (Bose and Som, 1986).

The average yield is 6.0 t/ha in 2003-04 (BBS, 2005). The yield potential of eggplant is low in Bangladesh compared to other countries. Of many reasons for high price of eggplant, lower production rate is important. Incidence of insect pests and diseases greatly hampered the production of eggplant. This crop suffers from the various diseases; about 13 different diseasse so far recorded in Bangladesh (Das *et al.* 2000; Khan *et al.* 1998 and Rashid, 2000). Among those diseases wilt of eggplant has been treated as one of the major constrains in eggplant cultivation in the country (Ali, 1993). Eggplant cultivation in Bangladesh is severly impaired by three important wilt causing pathogens viz. *Pseudomonas solanacearum*, *Fusarium oxysporum* and *Meloidogyne incognita*, the causal agent of Bacterial wilt, Fusarium wilt Nemic wilt, respectively and caused considerable damage of eggplant (Timm and Ameen, 1960; Talukder, 1974; Ahmed and Hossain, 1985; Mian, 1986 and Ali *et al.*, 1994). These are also the major limiting factors for eggplant production throughout the world (Hinata, 1986). Wilt problems are especially severe in the humid tropics. In some cases 100% of the plants are found to die in Kitchen gardens of Bangladesh due to wilt problem (Ali *et al.*, 1994).

The Fusarium wilt and Nemic wilt are very acute in Bangladesh. In recent year, bacterial wilt has become a great problem for eggplant cultivation in Bangladesh (Rashid, 1976). Screening of germplasms of eggplant to evaluate their resistance against *R. solanacearum* have immense value for the management of the disease in this country. Seveal workers attempted to search for bacterial wilt resistant varieties of eggplant (Hoque *et al.* 1981, Rahman and Hoque 1986, Khan 1974, Hossain *et al.* 1991). For the management of such important bacterial, Fusariam and Nemic diseases a few evidence of research work exists in Bangladesh. Search of resistant germplasms for the management of crop disease is considered as a ecofriendly approach. Besides, the use of chemicals for controlling soil borne pathogen like wilt pathogens are very costly to the growers. Thus the experiment was undertaken to screen out the resistant germplasm against Bacterial (*R. solanacearum*), Fungal (*Fusarium oxysporum*), Nemic (*Meloidogyne spp*) wilt of eggplant.

Materials and Methods

Experimental site

The experiment was conducted in the Field of SAU (Sher-e-Bangla Agricultural University) farm allotted for the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The experiment was carried out during the period form September 2005 to April 2006. The soil of the experimental plot was loam to clay loam in texture belonging to the Madhupur Tract (AEZ-28). The soils of the site were non-calcareous with loam to clay loam in texture.

Climate

The climate of the experimental area was of sub-tropical in nature characterized by high temperature associated with heavy rainfall during Kharif season (April to September) and scanty rainfall with moderately low temperature during Rabi season (October to March).

Eggplant variety used

Eggplant varieties Nayantara, Kazla, Uttara, Singhnath, Dhundul, Katabegun, Marichbegun, Luffa-s were used in the experiment for screening against wilt diseases. Healthy, mature and disease free seeds of eggplant varieties were collected from different sources like IPM Lab of Bangladesh Agricultural University, Bangladesh Agricultural Research Institute, and local market of eggplant growing areas on the second week of September. Ten grams of Healthy seeds were collected for each variety

Treatments of the experiment and design

In this study eight (8) varieties were used treatments were used as designated by T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8 which were as follows: 1) T_1 = Noyantara; 2) T_2 = Shingnath; 3) T_3 = Dhundul; 4) T_4 = Kazla; 5) T_5 = Marich Begun; 6) T_6 = Luffa; 7) T_7 = Kata Begun and; 8) T_8 = Uttara. The experiment was laid out in Randomized Block Design (RBD) with three replications. The whole plot was divided into three blocks each containing eight (8) plots of 3.5m x 1.0m size giving 24 unit plots. Each of the treatment put once at each block. The space kept between the blocks was 1m wide and between plots it was 0.5m.

Crop husbandry

Then the land was well-prepared before planting. Manure and fertilizers were applied as per standard recommendation. The following doses were used for carrying out the field experiment. After preparation of main pot in the net house 45 days old seedling were be uprooted from the seedbed and transplanted in the experimental pot on the 16th November 2005 in the afternoon of the same day. A sufficient irrigation was given just after transplantation with the help of a bucket sprinkler. Shading and watering was continued till the seedlings were established in the field. Weeding,

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gap filing, manuring and pest control were done as per requirements.

Inoculation of *Fusarium oxysporum*

Fusarium oxysporum was grown on PDA (Potato Dextrose Agar) medium at 25 ^{0}C temperature. After sporulation (15-20 days), added 5-ml/plate sterile water and the spore masses scraped away with sterile needle/scalpel. The conditional suspension thus made with additional water was then blended in a Moulinex blender for 2 minutes in medium speed and filtered through sterile cheesecloth, adjusted concentration 1.2 × 10^7 conidia/ml solution. Then inoculation done at the root zone of plant by drenching of spore suspension @ 250ml/plant with the help of compressed air hand sprayer following pulverized the soil to mix up the *Fusarium oxysporum* spores thoroughly to the soil. Inoculation done at 30 Days after transplanting (DAT).

Inoculation of *Meloidogyne incognita*

Mature eggmass of nematode (*M. incognita*) was collected from severely galled roots of eggplants using fine forceps. After thorough rinsing with sterile distilled water the egg mass were transferred to 75 mm (diameter) petridishes containing 10ml sterilized distilled water and were incubated at 28-29° C for four days. Inoculum suspension of freshly hatched larvae was prepared in distilled water having about 500 larvae per ml. Inoculation was done by powering the inoculum containing 1000 nematode suspended in 2 ml of water into two depressions made on the surface of the soil near the root system of seedlings. Inoculation was done at 30 Days after transplanting (DAT).

Inoculation of *Ralstonia solanacearum*

Pure culture of *Ralstonia solanacearum* E.F. Sm. was prepared on TZC (Tetrazolium chloride) medium by isolating the organism from the eggplant showing typical symptoms of bacterial wilt. The identification of the isolate was confirmed by Cock's postulate. The sick bed was established by inoculating the soil with a mixture of *Ralstonia solanacearum* isolates. The population density of the bacteria in the bed prior to set up the experiments was estimated about 2.3 x 108 CFU/Ml of water by dilution plate method.

Isolation of *Fusarium*

The experimental plots were inspected routinely to observe the Fusarium wilted plant. To identify the pathogen, the diseased plants were collected from the field and were taken to the laboratory, The diseased stem were cut into small pieces (about 0.5-1 cm) from the vascular region of the stem and surface sterilized by dipping in 10% Sodium Hypochlorite solution for 2-3 minutes or $HgCl_2$ solution (0.01) for 30 second. The cut pieces were then washed in water at three times and were placed into PDA media in sterilized petridish with the help of sterile forceps and incubated at 25±1°C for 10-15 days. Later the pathogen was purified using hyphal tip culture method and grown on PDA media at 25±1°C for 2 weeks. Causal organisms were identified under stereobinocular and compound microscopes (Fig 1-2).

Fig. 1. Twenty six days old culture of *Fusarium oxysporum*

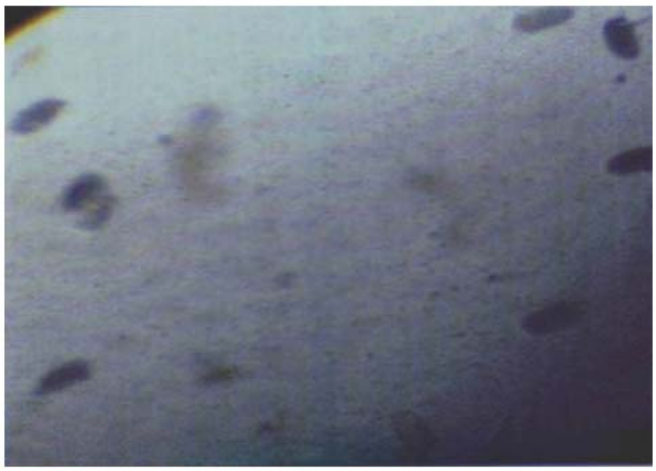
Fig. 2. Twelve days old culture o f *Fusarium oxysporum*

Isolation of Nematode

The experimental plots were inspected routinely to observe the Nematode wilted plant. To identify the nematode the diseased plants were collected from the field and were taken to the laboratory, and collected freshly soil sample of about 100-300cm^3 and the nematodes in it isolated by the Baermann funnel method. The funnel is placed on a stand and filled with water. Then soil sample, which is wrapped by 5-6 cm circular piece of cloth is placed in the funnel below the surface of the water and allowed them to stand overnight or for several hours. The active nematodes migrate through the cloth into the water and sink to the bottom of the water just above pinch up. 90% of the live nematode are recovered in the first 5-8ml of water drown from the rubber tubing. After extraction the water sample containing nematodes is placed in a dish for examination. Causal organisms were identified under stereo binocular and compound microscopes to find out nematode egg and adult (Fig. 3-4).

Fig. 3 Infected root of eggplant caused by *Meloidogyne* sp.

Fig. 4 Eggmass of *Meloidogyne incognita* in a crush gall of eggplant root (X 400)



The experimental plots were inspected routinely to observe the Bacterial wilted plant. To identify the pathogen, the diseased plants were collected from the field and were taken to the laboratory, The diseased stem were cut into small pieces from the vascular region of the stem and surface sterilized by dipping in 10% Clorox solution for different durations. With sterile forceps rinsed tissue section in distilled water and bloted on sterile paper towel. Then placed tissue pieces in tube of sterile water and macerated and made serial dilution by transferring 1 ml of bacterial suspensions from one tube to another next. After then placed 0.5 ml of each dilution into separate petridishes. Added melted but cooled agar stired gently and let solidified a few days single colonies appeared at one or more of the plates. Single colonies was subcultured bacteria was identified under microscope (Fig. 5-6).

Fig. 5. Four days old culture of *Ralstonia solanacearum*

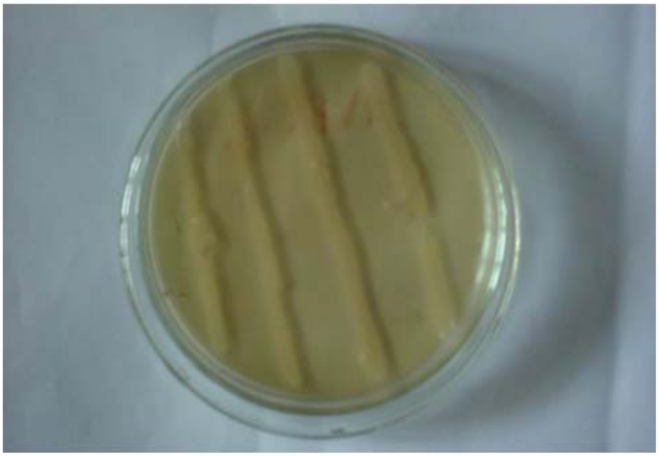


Fig. 6. Eighteen days old culture of *Ralstonia solanacearum*

Data recording and harvesting

Data on incidence of wilts were recorded in case of Bacterial wilt at 25, 35, 45, and 55 days after transplanting and Fusarium and Nematode wilt at 50, 65, 80, and 95days after transplanting by observation of visual symptoms. The disease incidence was calculated by the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of infected pants}}{\text{Number of total inspected plants}} \times 100$$

Analysis of data

The data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments. Treatment means were compared by DMRT (Duncan's Multiple Range Test). Correlation and Regression study was done to establish relationship between shoot length, shoot weight, root length, root weight with galling incidence among the treatments.

Results and Discussion

Bacterial wilt incidence

The reactions of different cultivars of eggplant in terms of bacterial wilt incidence significantly differed under the present experiment recorded at different days after transplanting (DAT) of eggplant. The variation of wilt incidence for different cultivar varied within a certain range for all the DAT.

Eggplant cultivar showed statistically significant variation in respect of Bacterial wilt incidence under the present trail at 25 DAT. All the cultivars performed comparatively lower wilt incidence at 25 DAT and the average wilt incidence varied from 20.00% to 41.00% (Table 1). The highest Bacterial wilt incidence (41.00%) was recorded in the cultivar Luffa which was statistically identical with the cultivar Kazla (40.00%). The lowest wilt incidences (20.00%) were recorded in the cultivar Singhnath and Kata Begun which was closely followed by the cultivar Dhundul and Uttara (30.00%). According to grading followed the variety D.R. Chowdhury gave resistant reaction with 13.3% wilting, variety Khatkhatia (long) was moderately resistant with 33.3% wilting and the rest of the varieties showed susceptible reaction.

Statistically significant variation was recorded among the eggplant cultivars in terms of Bacterial wilt incidence under the present trail at 35 DAT. All the cultivars performed comparatively medium level of wilt incidence at 35 DAT and it varied from 30.00% to 51.00 cm (Table 1). In the cultivar Luffa the highest bacterial wilt incidence (51.00%) was recorded which was statistically identical with the cultivar Kazla and Uttara (50.00%). The lowest wilt incidences (30.00%) were recorded in the cultivar Kata Begun which was closely followed by the cultivar Dhundal and Uttara (30.00%).

In the present experiment eggplant cultivar showed statistically significant variation in respect of Bacterial wilt incidence under the present trail at 45 DAT. All the cultivars performed comparatively higher wilt incidence at 45 DAT and the average wilt incidence at 45 DAT varied from 30.00% to 60.67% (Table 1). The highest Bacterial wilt incidence (60.67%) was recorded in the cultivar Nayatara and Luffa which was statistically identical with the cultivar Singhnath, Kazla and Marich Begun (40.00%). The lowest wilt incidences (30.00%) were recorded in the cultivar Kata Begun which was closely followed by the cultivar Dhundul and Uttara (50.00%). Hoque *et al.,* (1981) screened 16 eggplant varieties and the first symptom of wilting was observed on the 15th days after inoculation. Data on wilting collected after 43 days of inoculation were varied from 13.3 to 100.

A statistically significant variation was recorded among the eggplant cultivars in terms of Bacterial wilt incidence under the present trail at 55 DAT. All the cultivars executed comparatively higher level of wilt incidence at 55 DAT and it varied from 30.00% to 80.00 cm (Table 1). In the cultivar Luffa, the highest bacterial wilt incidence (80.00%) was recorded. The lowest wilt incidences (30.00%) was recorded in the cultivar Kata Begun which was closely followed by the cultivar Dhundul and Marich Begun (60.00%). Anonymous (1987) screened and eggplant varieties against Bacterial wilt disease and found that the percentage of wilting ranged from 49.9 to 95.5 %. Among the tested varieties none was found resistant.

Table 1. Bacterial wilt incidence at different stages of plant growth on different eggplant varieties

Treatments	Bacterial wilt incidence (%)			
	25 DAT	35 DAT	45 DAT	55 DAT
Nayantara	30.67 b	40.67 c	60.67 a	70.00 b
Singhnath	20.00 c	40.00 c	60.00 a	70.00 b
Dhundul	30.00 b	40.00 c	50.00 b	60.00 c
Kazla	40.00 a	50.00 b	60.00 a	72.33 b
Marich Begun	20.00 c	44.00 c	60.67 a	60.00 c
Luffa	41.00 a	51.00 b	60.00 a	80.00 a
Kata Begun	20.00 c	30.00 d	30.00 c	30.00 d
Uttara	30.00 b	50.00 b	50.00 b	60.67 c
$LSD_{(0.05)}$	1.33	1.33	1.046	2.651
Level of Significance	**	**	**	**
CV (%)	2.62	4.45	5.05	7.86

In a column means having similar letter(s) or without letter are identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

On the basis of wilt incidence different cultivars of eggplant were categorized into different groups mentioning their susceptible reactions to bacterial wilt (Table 2). Among the cultivars used in this trail only the cultivar Kata Begun was resistant to bacterial wilt whereas cultivar Marich Begun and Dundul were moderately resistant. On the other hand cultivar Kazla and Luffa were moderately resistant to bacterial wilt whereas Nayantara, Singhnath and Uttara were the moderately susceptible.

Table 2. Reaction of eggplant cultivar to bacterial wilt at 55 DAT

Treatments	Bacterial wilt incidence (%)	Reaction
Nayantara	70.00	Moderately susceptible (MS)
Singhnath	70.00	Moderately susceptible (MS)
Dhundul	60.00	Moderately resistant (MR)
Kazla	72.33	Susceptible (S)
Marich Begun	60.00	Moderately resistant (MR)
Luffa	80.00	Susceptible (S)
Kata Begun	30.00	Resistant (R)
Uttara	60.67	Moderately susceptible (MS)

Resistant (30% and below wilted plant); Moderately resistant (31%-50% wilted plant); Moderately susceptible (51%-70% and wilted plant); Susceptible (71% and above wilted plant). (Rashid *et. al.,* 2004)

Fusarium + Nematode wilt incidence (%)

The reactions of different cultivars of eggplant in terms of Fusarium + Nematode wilt incidence significantly differed under the present trail recorded at different days after transplanting (DAT) of eggplant. The variation of wilt incidence for different cultivar varied with in the certain range for all the DAT in all cultivars.

In the present experiment eggplant cultivar showed statistically significant variation in respect of Fusarium + Nematode wilt incidence at 50 DAT. All the cultivars performed comparatively lower wilt incidence at 50 DAT and the average wilt incidence at 50 DAT varied from 20.00% to 50.00% (Table 3). The highest Fusarium + Nematode wilt incidence (50.00%) was recorded in the cultivar Luffa which was statistically identical with the cultivar Dhudul (40.00%). The lowest wilt incidence (20.00%) was recorded in the cultivar Kata Begun which was identical with the cultivar Uttara (20.33%).

Eggplant cultivar showed statistically significant variation in terms of Fusarium + Nematode wilt incidence under the present trail at 65 DAT. All the cultivars performed comparatively medium wilt incidence at 65 DAT and the average wilt incidence varied from 30.00% to 60.00% (Table 3). The highest Fusarium + Nematode wilt incidence (60.00%) was recorded in the cultivar Luffa and Kazla which was statistically identical with the cultivar Singhnath, Dhudul and Marich Begun (50.00%). The lowest wilt incidence (30.00%) was recorded in the cultivar Kata Begun which was closely followed by the cultivar Nayantara (40.33%) and Uttara (40.33%).

In the present experiment eggplant cultivar showed statistically significant variation in respect of Fusarium + Nematode wilt incidence at 80 DAT. All the cultivars performed comparatively higher wilt incidence at 80 DAT and the average wilt incidence varied from 30.00% to 80.00% (Table 3). The highest Fusarium + Nematode wilt incidence (80.00%) was recorded in the cultivar Luffa which was closely followed by Kazla (70.00%). The lowest wilt incidence (30.00%) was recorded in the cultivar Kata Begun which was identical with the cultivar Nayantara and Uttara (50.33 and 50.67%, respectively).

Fusarium + Nematode wilt incidence on eggplant cultivars showed statistically significant variation under the present trail at 95 DAT. All the cultivars performed comparatively higher wilt incidence at 95 DAT and the average wilt incidence varied from 30.00% to 80.00% (Table 3). The highest Fusarium + Nematode wilt incidence was recorded in the cultivar Luffa (80.00%) and the lowest wilt incidence was recorded in the cultivar Kata Begun (30.00%) which was closely followed by the cultivar Uttara (57.00%).

Table 3. Fusarium and Nematode wilt incidence at different stages of growth on different eggplant varieties

Treatments	Fusarium + Nematode wilt incidence (%)			
	50 DAT	65 DAT	80 DAT	95 DAT
Nayantara	30.00 c	40.33 c	50.33 d	70.00 b
Singhnath	30.00 c	50.00 b	60.00 c	50.67 e
Dhundul	40.00 b	50.00 b	60.00 c	70.00 b
Kazla	30.33 c	60.00 a	70.00 b	70.00 b
Marich	30.00 c	50.00 b	60.00 c	60.00 c
Luffa	50.00 a	60.00 a	80.00 a	80.00 a
Kata	20.00 d	30.00 d	30.00 e	30.00 f
Uttara	20.33 d	40.33 c	50.67 d	57.00 d
$LSD_{(0.05)}$	0.522	0.545	0.821	1.562
Level of Significance	**	**	**	**
CV (%)	3.85	7.63	4.85	6.58

In a column means having similar letter (s) or without letter are identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

On the basis of Fusariun + Nematode wilt incidence, different cultivars of eggplant were categorized into different groups mentioning susceptible reactions (Table 4). Among the cultivars used in this screening program, only cultivar Kata Begun was found resistant to Fusarium + Nematode wilt. On the other hand cultivar Kazla was susceptible to Fusariun + Nematode wilt whereas Nayantara, Singhnath, Dhundul, Kazla, Marich Begun and Uttara were the moderately susceptible.

Table 4. Reaction of eggplant cultivars to Fusariun + Nematode wilt at 95 DAT

Treatments	Fusariun + Nematode wilt incidence (%)	Reaction
Nayantara	70.00	Moderately susceptible (MS)
Singhnath	50.67	Moderately susceptible (MS)
Dhundal	70.00	Moderately susceptible (MS)
Kazla	70.00	Moderately susceptible (MS)
Marich Begun	60.00	Moderately susceptible (MS)
Luffa	80.00	Susceptible (S)
Kata Begun	30.00	Resistant (R)
Uttara	57.00	Moderately susceptible (MS)

Resistant (30% and below wilted plant); Moderately resistant (31%-50% wilted plant); Moderately susceptible (51%-70% and wilted plant); Susceptible (71% and above wilted plant). (Rashid *et. al.,* 2004)

Considering the overall results it may be concluded that cultivar Kata Begun was graded as resistant against Bacterial, Fungal and Nemic wilt among the cultivars used in the experiment. However, further experiment need to conduct including more cultivars available in the country at different agro-ecological zone in the country.

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