

Management of Wilt Complex of Eggplant (*Solanum melongena* L.) Caused by *Fusarium oxysporum*, *Ralstonia solanacearum* and *Meloidogyne* spp.

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Abstract

Wilt complex disease of eggplant is a severe problem in Bangladesh as well as in the world. The pathogenic variability and their survival nature make the disease complex. The pathogen includes a fungus (*Fusarium oxysporum*), a bacterium (*Ralstonia solanacearum*) and a nematode (*Meloidogyne* spp.) that attack the plant individually or combinedly. The present investigation aimed to evaluate the integrated effects of different chemicals, poultry manure and *Trichoderma harzianum* to manage the disease. Carbendazim (Autostin 50WP), Streptomycin sulphate 9% and Tetracycline hydrochloride 1% (Krosin 10SP) and Carbofuran (Furadan 3G) were used as a fungicide, bactericide and nematicide, respectively which were integrated with poultry manure and *Trichoderma harzianum* resulting in sixteen treatments. The treatment efficacy varied significantly in comparison to control in respect of wilt incidence, yield and yield contributing characters. No wilt incidence was observed where *Trichoderma harzianum* was applied individually or in combination with Furadan 3G, Krosin 10SP, Autostin 50WP and poultry manure. The highest yield increase (300%) over control was noted in the treatment where Krosin 10SP, Furadan 3G, *Trichoderma harzianum* and Poultry manure were applied combinedly. The same treatment showed the best performance over control by 32%, 29%, 60%, 53% in case of the number of branches, number of leaves, number of fruits and fruit length, respectively. This treatment (Krosin 10SP + Furadan 3G + *Trichoderma sp.* + Poultry manure) also provided the highest Benefit Cost Ratio (5.68).

Keywords

Eggplant, Integrated Management, *in Vitro* Evaluation, Bioagent, Wilt Complex Disease

1. Introduction

Eggplant (*Solanum melongena* L.) is one of the most important and highly productive vegetable crops in the tropical and sub-tropical region of the world. It is cultivated widely in Indian sub-continent for its year-round availability, food value, taste, cash crop and also as an export commodity. It is the second most important vegetable crop in Bangladesh with respect to acreage and production [1]. However, production and yield rate of eggplant in Bangladesh is abysmal compared to other countries like India, Japan, China, etc., due to infection by different diseases. Eggplant cultivation is significantly impaired by wilt complex caused by *Fusarium oxysporum*, *Ralstonia solanacearum* and *Meloidogyne spp* [2] [3]. Nematodes facilitate fungi and bacteria to cause infection by creating wounds. The fungus and bacteria infect the vascular system of the plant and disrupt water and nutrient transportation resulting in wilt incidence. Nemic wilt shows knot/gall formation in the root system and the plants become weak due to the hindrance of water and nutrient uptake. The interaction of fungus, bacteria and nematode is called wilt complex and could be seen even in the same plant.

Farmers often face substantial crop loss due to the wilt complex resulting in severe economic losses. In case of bacterial wilt, 100% of the affected plants may collapse in the kitchen gardens of Bangladesh [4] while the severity is 10% - 90% in field conditions [5] [6]. For fungal wilt, the fruit yield is reduced by 20% - 30% [7], and it may turn into epidemic by complete crop failure in the winter season [8]. Nemic wilt (*Meloidogyne spp.*) solely may cause 27.2% yield loss of eggplant [9], and it may rise to 80% in solanaceous crops [10].

The standard control measures against wilt complex include the use of resistant varieties, crop rotation, agronomic practices, biological control and integrated management [11]. However, cultural practices and crop rotation may provide limited control of *Ralstonia solanacearum* [12] [13]. Discriminate uses of chemical pesticides create a harmful effect on the environment and ecology that leads the eggplants with residual effects [14]. Due to this, the export of eggplants from Bangladesh is restricted, which is alarming for the host country [15].

Integration of plant health management strategies (soil amendments, biocontrol agent and judicious use of fungicide, bactericide and nematicides) is now considered an effective approach emphasizing the environment, economics and social acceptance compared to conventional management. *Trichoderma spp.* is a well-documented biocontrol agent against a lot of soil and seed-borne pathogens for its antagonism, mycoparasitism and competition [16] [17] [18]. *T. harzia-*

num and *T. viride* reported complete inhibition of mycelial growth of *Fusarium oxysporum* [19]. Bactericides and biocontrol agents are reported effective against *Ralstonia solanacearum* [20]. Organic residues supply plant nutrients and increase natural suppressiveness of the soil against soil-borne pathogens and improve physio-chemical and biological characteristics [21]. Suppression of *Ralstonia solanacearum* is seen in poultry manure amended soil [22]. Carbofuran is the most effective nematicide in controlling *Meloidogyne* sp. in warm weather conditions. Moreover, integration of poultry manure, bioagent and carbofuran considerably reduce gall index and give higher fruit yield over control [23]. The present research work has been designed to formulate an integrated approach using those eco-friendly options for the management of wilt complex of eggplant.

2. Materials and Methods

2.1. Isolation and Identification of Pathogens

Fusarium oxysporum and *Ralstonia solanacearum* were isolated from the infected stem of eggplant, and *Meloidogyne* spp. was isolated from the knots of the infected root. *F. oxysporum* was purified by hyphal tip culture method from the diseased stem by blotter method and was identified by the key characters (CMI) (Figure 1). The bacterial wilt of an infected plant was confirmed by bacterial ooze test by making the water turbid. From the serial dilution of ooze suspension, 100 μ L of each concentration was poured followed by spreading with a sterilized spreader onto the surface of the solidified TTC media. The inoculated plates were incubated at 32°C for 48 hours. The isolated bacterium was streaked on CPG and NA media and was identified as *R. solanacearum* by morphological and colony characters [24] and gram staining [25] (Figure 2). The roots of nemic wilted plant were washed gently under running tap water. A branch of root bearing knots was examined under a stereomicroscope, and a number of egg masses were noticed. A single egg mass of the root knot was picked up in semi-permanent slide and crushed for studying under the compound microscope. An adult pear-shaped female nematode was pulled from the hole which was seen after removing an egg mass and identified as *Meloidogyne* spp. By

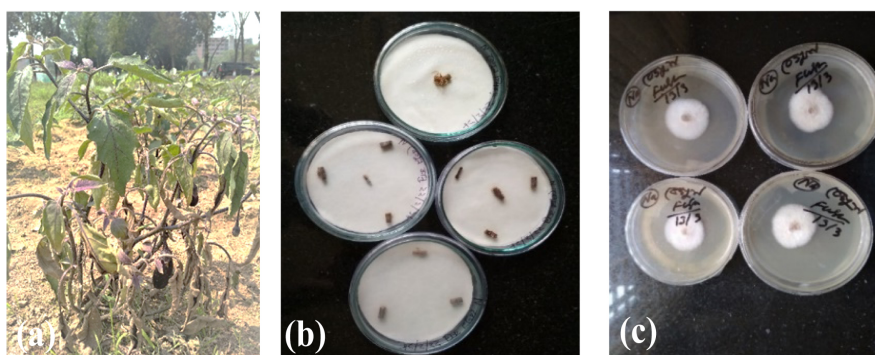


Figure 1. Isolation and preparation of pure culture of *Fusarium oxysporum* from infected stem. (a) Symptom of Partial wilted eggplant; (b) Placing infected root in blotter paper; (c) Purification of fungus through hyphal tip culture.

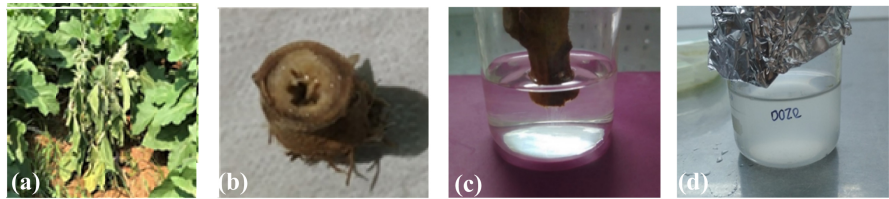


Figure 2. Isolation and preparation of pure culture of *Ralstonia solanacearum* on TTC media from infected stem by dilution plate technique. (a) Symptom of bacterial wilt; (b) Brown discoloration at vascular system with ooze; (c) Milky white ooze in water; (d) Ooze suspension.

observing under stereo and compound microscope (**Figure 3**).

2.2. *In Vitro* Assay of Fungicide and Bactericide

The fungicide Autostin 50WP (Carbendazim) (Supplementary **Table 1**) was assayed against *Fusarium oxysporum* by Food Poisoning Technique, cup or groove method [26]. Five mm disc of PDA media in each petri plate was scooped from 3 places maintaining an equal distance from the centre using a sterilized disc cutter. After putting Autostin 50WP (0.1%) solution into each hole, the plates were kept overnight for the diffusion of fungicide into the medium. 5 mm mycelial discs of 7 days old culture of *Fusarium oxysporum* were taken and placed at the centre of the diffused PDA plates. The plates with holes filled with sterile water were served as control. All the plates were incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for seven days, and mycelial growth of *Fusarium oxysporum* was recorded. The percent inhibition of mycelial growth was calculated using the formula used by Islam [26] and [27]. Bioassay of antibacterial Krosin 10SP (Streptomycin sulphate 9% and Tetracycline hydrochloride 1%) (Supplementary **Table 1**) against *Ralstonia solanacearum* was done by zone inhibition method. 10 ml nutrient broth in a test tube was inoculated with 48 hours old pure culture of bacteria grown on NA media plate. After shaking in a shaker incubator at 30°C and 150 rpm for 24 hours, the broth culture was spread uniformly on TTC media plate using the sterile cotton swabs. 5 mm disc was scooped and filled with Krosin 10SP suspension (0.05%) while inoculated plates filled with sterile water was treated as control. The plates were incubated at 30°C in incubation chamber for 48 hours, and the diameter of the inhibition zone was measured.

2.3. Pathogenicity Test for the Causal Pathogens

The root-dip assay modified from Ramaiah and Garampalli [19] was followed for pathogenicity test of *Fusarium oxysporum*. Seedlings with wounded roots were submerged for 10 min in the conidial suspension ($1 \times 10^6 \text{ ml}^{-1}$) obtained from 7 days old culture of *Fusarium oxysporum* on. In the case of *Ralstonia solanacearum*, 5 ml spore suspension (10^5 CFU ml^{-1}) was injected into the vascular system of the 25 days old seedlings (**Figure 4**). The plants expressing wilt symptoms were selected. The fungus and bacterium were re-isolated from the symptom and compared with the previous culture of *Fusarium oxysporum* and

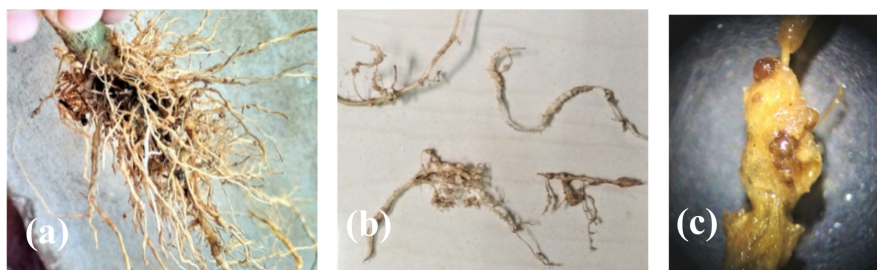


Figure 3. Isolation and preparation of culture of *Meloidogyne* spp. (a) and (b) Roots showing knots; (c) Egg masses on a knot.



Figure 4. Inoculation of *Ralstonia solanacearum* suspension by injection.

Ralstonia solanacearum, respectively to satisfy the Koch's postulates. Egg masses were picked up from the roots, kept for hatching in water for 24 to 36 hours. The hatched second stage (J2) juveniles were allowed to inoculate 21 days old seedlings of eggplant. After 100 to 150 days when the nematodes complete 2 - 3 generations, the root galls were explored for confirmation of the causal nematode. In each case, uninoculated plants served as a positive control.

2.4. Integrated Experiment

Thirty-five days old seedlings of BARI hybrid begoon 3 (oblong) were transplanted in the research field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh during September, 2017. Sixteen treatments were arranged with 3 replications in Randomized Complete Block Design (RCBD) where each plot size was 3 m² (3.0 m × 1.0 m) containing 5 plants per plot with 0.6 m × 0.6 m planting distance. Every treatment was placed once at each block. Treatments were T₁ = Autostin 50 WP (seedling treatment + foliar spray @ 0.2%); T₂ = Furadan 3 G (Supplementary **Table 1**) (soil treatment @ 10 g pit⁻¹); T₃ = Krosin 10SP (seedling treatment + drenching of rhizosphere soil @ 0.1%); T₄ = *Trichoderma* formulation (Soil application @ 20 g·m⁻²: 1 × 10⁸ cfu g⁻¹ of soil); T₅ = Poultry manure (soil amend-

ment @ 2 kg 3 m⁻²; T₆ = Autostin 50 WP + *Trichoderma* formulation; T₇ = Furadan 3 G + *Trichoderma* formulation; T₈ = Krosin 10SP + *Trichoderma* formulation; T₉ = Autostin 50 WP + Poultry manure; T₁₀ = Furadan 3 G + Poultry manure; T₁₁ = Krosin 10SP + Poultry manure; T₁₂ = *Trichoderma* formulation + Poultry manure; T₁₃ = Autostin 50 WP + *Trichoderma* formulation + Poultry manure; T₁₄ = Furadan 3 G + *Trichoderma* formulation + Krosin 10SP; T₁₅ = Krosin 10SP + Furadan 3 G + *Trichoderma* formulation + Poultry manure; T₁₆ = Control.

2.5. Application of Treatments

Autostin (0.2%) and Krosin (0.1%) solutions were applied for seedling-root treatment before transplanting, followed by foliar spraying. Spraying of treatments were done 3 times with 10 days' interval after transplanting. Furadan 3G was applied in pit soil (5 gm pit⁻¹) during transplanting of seedlings. Poultry manure was applied @ 2 kg plot⁻¹ as a soil amendment before 21 days of transplanting to the allotted plots. Spore suspension of *Trichoderma harzianum*. was prepared by scraping the 10 - 12 days old culture, maintaining the concentration 1 × 10⁸ conidia ml⁻¹ solution. The roots of seedlings were soaked with *Trichoderma* suspension. The specific pit soil was treated with black gram based *Trichoderma* formulation (50 gm pit⁻¹) where Black gram: peat soil: Cowdung = 1:2:1.

The Disease Incidence or severity was measured, and costing for estimation of Benefit Cost Ratio (BCR) was done as exercised by Islam *et al.* [28] (Supplementary **Table 2** and **Table 3**).

$$\% \text{ Disease Incidence/Severity} = \frac{\text{Number of infected plant(s)}}{\text{Number of total plant(s)}} \times 100$$

$$\text{BCR} = \text{Gross return (Tk./ha)} \div \text{Total cost of production (Tk./ha)}$$

3. Results

3.1. Identification of *Fusarium Oxysporum*, *Ralstonia Solanacearum* and *Meloidogyne* Spp.

Initially, *F. oxysporum* produced white cottony mycelium on PDA media. The fungus grew slowly and with time made pink pigmentation (**Figure 5**). Several-celled and slightly curved, hyaline macro conidia were observed in PDA culture. Single-celled, hyaline, ovoid to globose micro conidia were also found from 21 days old pure culture of *Fusarium oxysporum*. In case of bacteria, colonies were appeared as the red coloured centre with whitish margins (virulent) within 24 - 48 hours culturing on semi selective media Tetrazolium chloride (TZC) medium. While culturing on NA media, circular, mucoid, convex, lucid coloured colonies were found (virulent). The bacterium was rod-shaped with rounded ends and gram-negative (red colour) under the compound microscope at 1000X magnification with oil immersion objective. These characteristics were matched

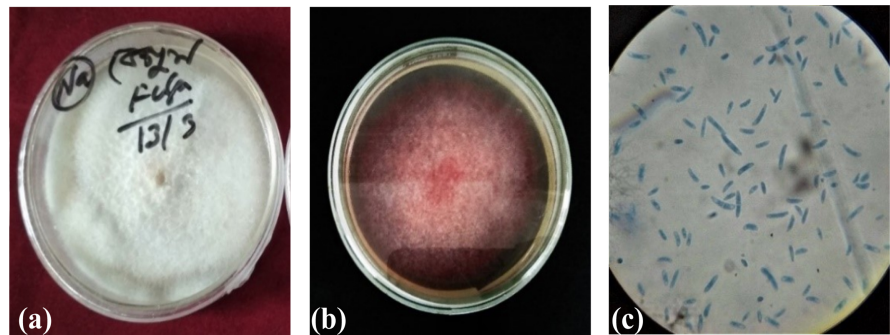


Figure 5. Morphological features of *Fusarium oxysporum*. (a) Pure culture of *Fusarium oxysporum* showing whitish mycelial growth on PDA media; (b) Pure culture of *F. oxysporum* showing pink discoloration on PDA media; (c) Macroconidia of *Fusarium oxysporum* under compound microscope 400 \times .

with *Ralstonia solanacearum* (Figure 6). In the case of nematode, pear-shaped female surrounded by egg mass were found under stereomicroscope which was prepared from root knot and identified as *Meloidogyne* spp. Eggs were found ellipsoid, lack of uniformity. Larvae and adults were also observed (Figure 7).

The findings of the present investigation coronated with the results of previous research with so much extent. Nelson *et al.* [29] and Joshi *et al.* [30] described that the colony appearances of *F. oxysporum* on PDA, which was highly variable. The pink colony colour and white cottony mycelium is formed on PDA media. Three types of asexual spores viz. macroconidia, microconidia and chlamydospore were produced. Macroconidia are slightly curved, sickle shaped and 4 - 5 celled where microconidia are round/globose and single-celled. Kumar & Sarma [31] and Dhital [32] narrated that *Ralstonia solanacearum* is a soil-borne aerobic, rod-shaped, non-spore-forming, gram-negative bacterium. Colonies of *Ralstonia solanacearum* appear as red coloured with whitish margins (virulent) on TTC medium which was highly fluidal. Circular, mucoid, convex, lucid coloured colonies were found on NA medium with respect to some samples. The pear-shaped female nematode of *Meloidogyne* sp. produced a huge number of knots or galls in the root system, and egg masses were attached with the knots [33].

3.2. Pathogenicity Test

The pathogenicity of *Fusarium oxysporum*, *Ralstonia solanacearum* and *Meloidogyne* spp. was satisfied the Koch's postulates (Figure 8). In *Fusarium oxysporum*, the inoculated plants showed wilt symptom after 20 - 30 days of post-inoculation. But in the case of bacteria, it was taken only one week for wilting. In contrast, the control plants were symptomless. On reisolation, the colonies were found to be similar to that fungus and bacteria inoculated previously. In the case of *Meloidogyne* spp., knots were found on the root system after 100 - 150 days of inoculation. Female nematode, egg masses and juveniles were similar to the previous *Meloidogyne* spp.

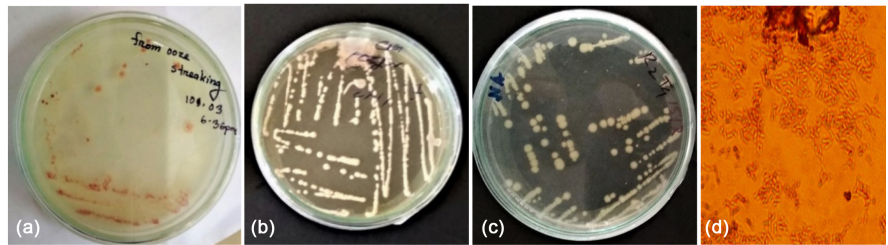


Figure 6. Morphological features of *Ralstonia solanacearum*. (a) *Ralstonia solanacearum* growing on semi selective TTC media giving pink coloured colonies with whitish margin; (b) Pure culture of *Ralstonia solanacearum* on CPG media; (c) Pure culture of *Ralstonia solanacearum* on NA media; (d) Rod shaped Gram negative *Ralstonia solanacearum* under oil emersion objective, total magnification 1000×.

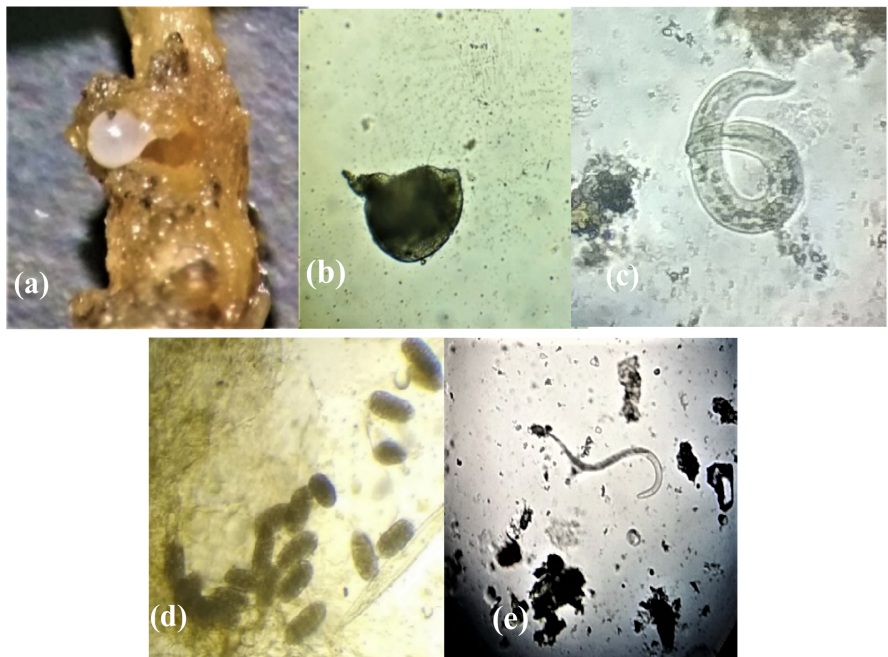


Figure 7. Morphological features of *Meloidogyne* spp. (a) Pear shaped female nematode under stereomicroscope; (b) Pear shaped female nematode under compound microscope; (c) Adult male nematode under compound microscope; (d) Eggs after crushing of egg mass; (e) Juvenile under compound microscope (400×).

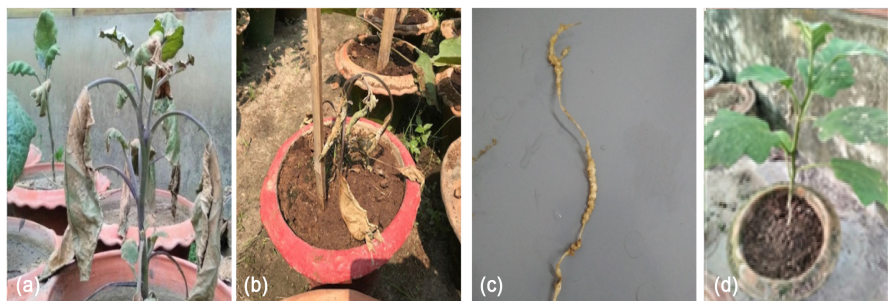


Figure 8. Pathogenicity test of targeted pathogens. (a) Wilted plant inoculated with *Fusarium oxysporum*; (b) Wilted plant inoculated with *Ralstonia solanacearum*; (c) Showing knot on root inoculated with *Meloidogyne* spp.; (d) Uninoculated healthy plant in control. N = 3 (corresponding to 3 independent biological replications).

3.3. Evaluation of Chemicals against Isolated Pathogens

The antipathogenic activity of different chemicals were evaluated using the poison plate method. The chemicals tested in the present study showed a gradual decline in growth of associated pathogens even in low concentration (**Figure 9**). Autostin 50 WP (0.1%) and Krosin 10SP (0.05%) exhibited potent antifungal and antibacterial activity against *Fusarium oxysporum* and *Ralstonia solanacearum*, respectively. Autostin 50WP (0.1%) and Krosin 10SP (0.05%) were found to be effective and gave 81% and 87% reduction of growth of *Fusarium oxysporum* and *Ralstonia solanacearum*, respectively (**Table 1** & **Table 2**).

Table 1. *In vitro* assay of Autostin 50 WP against of *Fusarium oxysporum*.

Treatments	Radial mycelial growth (cm)	% Inhibition over control = $\frac{\text{Radial growth of pathogen in (control - treated) plate}}{\text{Radial growth of pathogen in control plate}} \times 100$
Autostin 50 WP @ 1% (Carbendazim)	1.66 b	81.35
Control (Sterile water)	8.90 a	-
Lsd value		0.28
CV %		2.23

Table 2. *In vitro* assay of Krosin 10 SP against of *Ralstonia Solanacearum*.

Treatment	Inhibition zone (cm)	% Inhibition over control = $\frac{\text{Inhibition zone in (treated - control) plate}}{\text{diameter of the petridish}} \times 100$
Krosin AG 10SP @ 0.5% (Streptomycin sulphate + Tetracycline hydrochloride)	7.76 a	86.29
Control (Sterile water)	0.0 b	--
Lsd value		0.40
CV %		4.48

Autostin 50 WP was found to be promising in the reduction of the growth of *Fusarium oxysporum*. Amini *et al.*, [34] observed that Carbendazim (Autostin 50 WP) was effective in controlling *Fusarium oxysporum* in *in vitro* and field condition. Murakoshi and Takahashi [35] and Svetlana Milijasevic *et al.* [36] stated that antibiotics like Streptocyclin found to be very effective in controlling *Ralstonia solanacearum*, causing bacterial wilt. Krosin contains two types of active ingredient *i.e.* Streptomycin sulphate 9% and Tetracycline hydrochloride 1% that reduces the selection pressure and controls bacteria efficiently.

3.4. Effect of Different Treatments on Wilt Complex and Yield of Eggplant

The incidence of fungal wilt with the application of different treatments singly or

in combination varied sharply ranging from 0 to 13.33% against *Fusarium oxysporum*. Plants in control treatments showed the highest disease incidence (13.33%) while no wilt incidence was noticed in case of treatments T₁, T₄, T₅, T₆, T₇, T₈, T₉, T₁₂, T₁₃, T₁₄ and T₁₅ (Table 3). In case of incidence of bacterial wilt, treatment T₂, T₃, T₄, T₇, T₈, T₁₂, T₁₃, T₁₄ and T₁₅ exhibited complete reduction of *Ralstonia solanacearum* compared to control (33.33%). Nemic wilt incidence was also greater in control plots (13.33%) than in treatment T₁, T₆, T₉ and T₁₂ (6.67%) whereas no nemic wilt incidence was observed in case of treatment T₂, T₄, T₇, T₈, T₁₃, T₁₄ and T₁₅. The cumulative incidence wilt complex also had the similar trend where the maximum incidence of wilt complex was recorded in control (60%). In contrast, no wilt complex was noticed in case of treatment T₁₅ where Krosin, Furadan, *Trichoderma* formulation and Poultry manure were applied combinedly. The minimum yield was obtained in control treatment T₁₆ (8.02 t·ha⁻¹) (Table 3).

Table 3. Effect of different treatments on Wilt Incidence (DI) and yield of eggplant.

Treatments	Disease incidence (DI) (%)			Total Incidence of wilt complex (%)	Yield (ton hectre ⁻¹)
	<i>Fusarium</i> wilt	Bacterial wilt	Nemic wilt		
T ₁ = Autostin 50 WP	0.00 (0.71) b	6.67 (1.96) bc	6.67 (1.96) ab	13.33(3.21) bc	20.21 d-f
T ₂ = Furadan 3G	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc	19.02 ef
T ₃ = Krosin 10SP	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc	19.62 ef
T ₄ = <i>Trichoderma</i> sp	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c	25.86 bc
T ₅ = Poultry manure	0.00 (0.71) b	13.33 (3.22) b	6.67 (1.96) ab	20.00 (3.83) b	18.13 f
T ₆ = T ₁ + T ₄	0.00 (0.71) b	6.67 (1.96) bc	6.67 (1.96) ab	13.33(3.21) bc	20.80 d-f
T ₇ = T ₂ + T ₄	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c	19.32 ef
T ₈ = T ₃ + T ₄	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c	27.34 b
T ₉ = T ₁ + T ₅	0.00 (0.71) b	6.67 (1.96) bc	6.67 (1.96) ab	13.33(3.21) bc	19.91 d-f
T ₁₀ = T ₂ + T ₅	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc	18.43 f
T ₁₁ = T ₃ + T ₅	6.67 (1.96) ab	0.00 (0.71) c	0.00 (0.71) b	6.67 (1.96) bc	23.18 cd
T ₁₂ = T ₄ + T ₅	0.00 (0.71) b	0.00 (0.71) c	6.67 (1.96) ab	6.67 (1.96) bc	21.10 d-f
T ₁₃ = T ₁ + T ₄ + T ₅	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c	27.56 b
T ₁₄ = T ₂ + T ₄ + T ₃	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c	28.24 b
T ₁₅ = T ₂ + T ₃ + T ₄ + T ₅	0.00 (0.71) b	0.00 (0.71) c	0.00 (0.71) b	0.00 (0.71) c	32.10 a
T ₁₆ = Control	13.33 (3.21) a	33.33 (5.71) a	13.33 (3.21) a	60.00 (7.45) a	8.02 g
CV %	104	72.80	105.43	80.06	9.61

*Values in a column with same letter (s) do not differ significantly (p = 0.05). *Figures in parenthesis are the mean of square root transformed values.

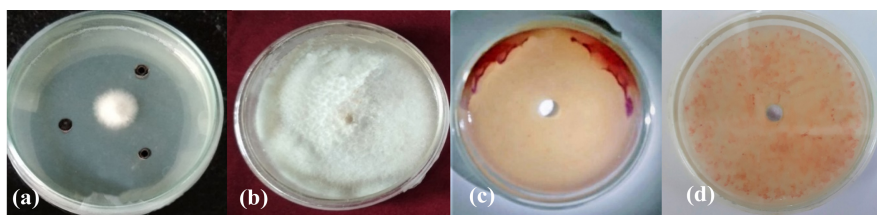


Figure 9. *In vitro* assay of selected chemicals by food poisoning method (Cup method). (a) Autostin 50WP treated plate against *Fusarium oxysporum* compared to control (b). (c) Krosin 10 SP treated plate against *Ralstonia solanacearum* compared to control (d).

3.5. Effect of Different Treatments on Plant Growth Parameters against Wilt Complex of Eggplant

The yield contributing characters of eggplants significantly influenced by the treatments applied against wilt complex. The highest shoot length (76.40 cm) was observed in T₅, whereas the lowest length of the shoot was in the control plants (53.97 cm). The number of branches (17.03) as well as leaves (179.0) was maximum in T₁₅ where *Trichoderma* formulation, Krosin, Furadan and Poultry manure were applied combinedly. The plants treated with T₁₅ produced the maximum number of fruits (37.33) while the lowest number of fruits plant⁻¹ (23.37) was recorded in the control plots. The highest fruit length (23.00 cm) was observed in case of treatment T₁₅ followed by the treatments T₁₃, T₁₄ and T₁ (Table 4).

3.6. Effect of Treatments on Growth Parameters and Yield Over Control

Treatment T₁₅ (Krosin 10SP + Furadan 5G + *Trichoderma* formulation + Poultry manure) was found to influence the number of branches, leaves, fruits, fruit

Table 4. Effect of different treatments on plant growth parameters against wilt complex of eggplant.

Treatments	Plant height (cm)	No. of branches plant ⁻¹	No. of leaf plant ⁻¹	No. of fruit plant ⁻¹	Fruit length
T ₁ = Autostin 50 WP	63.63 h	13.40 e	163.33 c-e	30.10 c-f	20.47 b-d
T ₂ = Furadan 3G	64.07 gh	14.93 cd	162.33 c-e	28.13 fg	19.20 c-e
T ₃ = Krosin 10SP	67.00 e-h	14.87 d	163.67 b-e	28.53 e-g	19.80 b-e
T ₄ = <i>Trichoderma</i> sp.	69.00 c-f	15.80 a-d	162.67 c-e	28.77 d-g	17.00 f-h
T ₅ = Poultry manure	76.40 a	15.10 cd	161.67 c-e	30.70 b-f	19.13 c-f
T ₆ = T ₁ + T ₄	71.53 b-d	16.13 a-d	156.33 e	28.87 c-g	18.07 e-g
T ₇ = T ₂ + T ₄	65.80 f-h	15.03 cd	158.00 de	26.47 g	18.53 d-g
T ₈ = T ₃ + T ₄	72.67 a-c	16.57 ab	163.67 b-e	31.57 b-e	17.00 f-h
T ₉ = T ₁ + T ₅	65.57 f-h	16.13 a-d	162.33 c-e	30.53 b-f	15.73 h
T ₁₀ = T ₂ + T ₅	68.33 c-g	15.70 a-d	162.00 c-e	28.47 e-g	16.60 gh
T ₁₁ = T ₃ + T ₅	67.07 e-h	16.23 a-c	168.00 b-d	31.97 b-d	18.07 e-g
T ₁₂ = T ₄ + T ₅	67.27 d-h	15.57 b-d	170.67 a-c	29.50 c-g	19.93 b-e
T ₁₃ = T ₁ + T ₄ + T ₅	72.33 a-c	16.93 a	174.33 ab	33.43 b	21.47 ab
T ₁₄ = T ₂ + T ₄ + T ₃	70.57 b-e	16.27 a-c	172.00 a-c	32.07 bc	20.93 a-c
T ₁₅ = T ₂ + T ₃ + T ₄ + T ₅	73.63 ab	17.03 a	179.00 a	37.33 a	23.00 a
T ₁₆ = Control	53.97 i	12.93 h	139.00 f	23.37 h	15.07 h
CV %	3.90	5.16	4.03	6.55	7.00

*Values in a column with same letter (s) do not differ significantly ($p = 0.05$).

length and fruit yield of eggplant by 32%, 29%, 60%, 53% and 300%, respectively. Poultry manure increased plant height by 42% over control where treatment T₁₅ increased 36% (Figure 10).

3.7. Benefit Cost Ratio (BCR)

The benefit cost ratio (BCR) of treatment T₁₅ (Krosin + Furadan + *Trichoderma* formulation + Poultry manure) was the highest (5.68) followed by T₁₄ (5.56) and T₄ (5.48) (Table 5).

Integrated approach by employing three chemicals viz. Autostin 50WP (Carbendazim), Furadan 3G (Carbofuran), and Krosin 10SP (streptomycin sulphate) along with a bio-agent *Trichoderma harzianum* and a soil amendment by poultry manure singly and in combinations were explored for the management of wilt complex of eggplant. The treatment combinations showed a better result

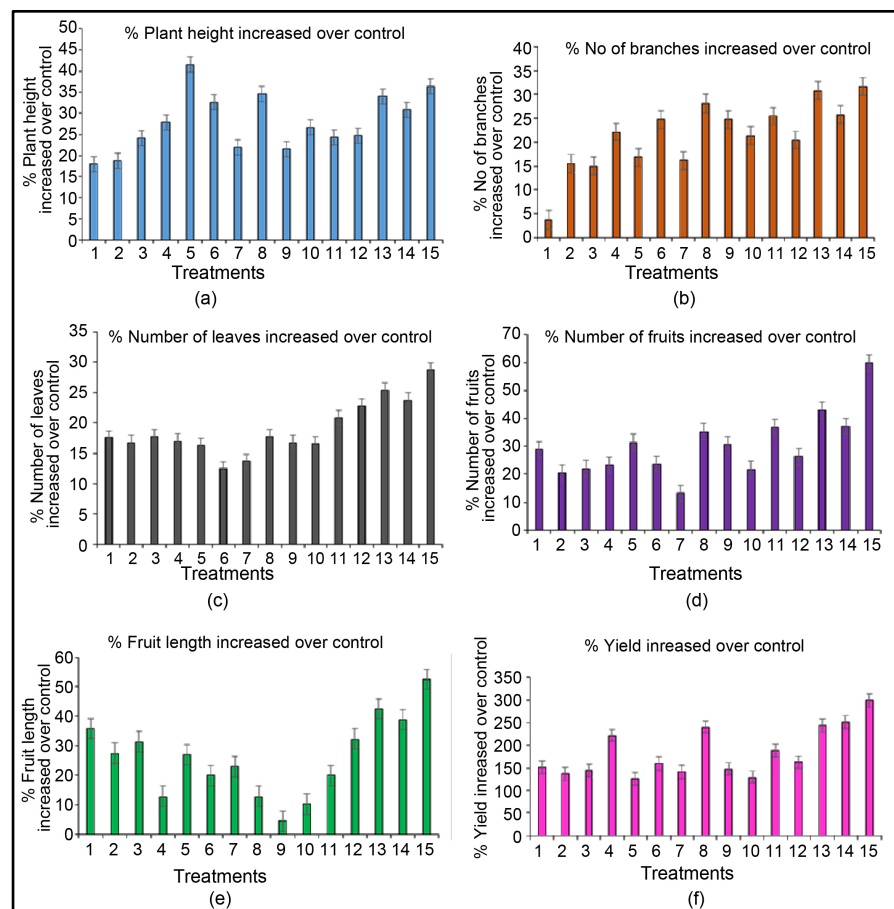


Figure 10. Effect of different treatments on growth parameters and yield over control. (a) % Plant height increased over control; (b) % Number of branches increased over control; (c) % Number of leaves increased over control; (d) % Number of fruits increased over control; (e) % Fruit length increased over control; (f) % Yield increased over control. Treatments: T₁ = Autostin 50 WP; T₂ = Furadan 3G; T₃ = Krosin 10SP; T₄ = *Trichoderma* formulation; T₅ = Poultry manure; T₆ = T₁ + T₄; T₇ = T₂ + T₄; T₈ = T₃ + T₄; T₉ = T₁ + T₅; T₁₀ = T₂ + T₅; T₁₁ = T₃ + T₅; T₁₂ = T₄ + T₅; T₁₃ = T₁ + T₄ + T₅; T₁₄ = T₂ + T₄ + T₅; T₁₅ = T₂ + T₃ + T₄ + T₅; T₁₆ = Control.

Table 5. Benefit Cost Ratio (BCR) of treatments for controlling wilt complex of eggplant.

Treatments	Yield (ton) ha ⁻¹	Gross return (Tk ha ⁻¹)	Total cost of production = Fixed cost + Treatment cost (Tk ha ⁻¹)	BCR
T ₁ = Autostin 50WP	20.21	404,280	97,420	4.14
T ₂ = Furadan 3G	19.02	380,500	100,720	3.77
T ₃ = Krosin 10SP	19.62	392,400	96,640	4.06
T ₄ = <i>Trichoderma</i> sp	25.86	517,240	94,320	5.48
T ₅ = Poultry manure	18.13	362,660	103,320	3.51
T ₆ = T ₁ + T ₄	20.80	416,180	100,020	4.16
T ₇ = T ₂ + T ₄	19.32	386,440	103,320	3.74
T ₈ = T ₃ + T ₄	27.34	546,980	99,240	5.51
T ₉ = T ₁ + T ₅	19.91	398,340	109,020	3.65
T ₁₀ = T ₂ + T ₅	18.43	368,620	112,320	3.28
T ₁₁ = T ₃ + T ₅	23.18	463,740	108,240	4.28
T ₁₂ = T ₄ + T ₅	21.10	422,120	105,920	3.98
T ₁₃ = T ₁ + T ₄ + T ₅	28.24	555,120	111,620	4.97
T ₁₄ = T ₂ + T ₄ + T ₃	22.29	602,100	108,240	5.56
T ₁₅ = T ₂ + T ₃ + T ₄ + T ₅	32.10	681,400	119,840	5.68
T ₁₆ = Control	8.02	160,520	91,720	1.75

*1 United States dollar = 84.83 Bangladeshi taka (Tk). *Costing was done on the basis of market price' 2018.

than the individual application of each of the management option except *Trichoderma* formulation. The combination of bactericide (Krosin 10 SP), nematocide (Furadan 3G), *Trichoderma* formulation and poultry manure showed promising performances satisfying the Benefit Cost Ratio (BCR). *Trichoderma* formulation and Poultry manure not only suppress the soil-borne pathogens but also improved soil properties which resulted in higher yield.

It is worth mentioning here that the combination of treatments without Autostin 50 WP also showed superior results in reducing the fungal wilt incidence. The possible reason might be the *Trichoderma* formulation which acted against *Fusarium oxysporum*, consequently controlled the fungal incidence and some cases can cause 95% recovery of infection [37]. It is assumed that nematocide controlled not only nematode but also bacteria. The farmers of Philippine noticed no wilt symptom even in infected sucker of banana caused by *Ralstonia solanacearum* after treating with Furadan (a.i. carbofuran 48%) [38]. For this mechanism, Furadan 3G was enabled to combat the bacteria, even in absence of bactericide in the treatment. As the nematode infection facilitates the penetration of bacteria through wounds, Furadan indirectly control the bacteria by managing nematode. Combined application of Carbofuran 3G, Neem cake, Streptocyclin and *Trichoderma harzianum* was effective in controlling *Meloidogyne incognita* and *Ralstonia solanacearum* causing wilt complex of eggplant under field condition [39]. The integration of Streptocyclin @0.5 g/L + COC 50% WP (1 g/L) in-

tercropped with Mustard cake was successful in reducing bacterial wilt incidence by 23.35% and formation of knots by 72.4% [40].

Trichoderma spp. enhance plant growth and productivity [41] [42]. The mechanisms of *Trichoderma* spp. were antagonism, mycoparasitism, competition with pathogens for nutrient and space and induction of systemic resistance in plants resulting in the reduction of disease and increase in yield. Carbofuran also showed a prominent influence on the growth parameters and suppression of *Meloidogyne* spp. and *Ralstonia solanacearum* [38] [43]. The addition of poultry manure in the treatments ensued the increased yield by boosting up the soil fertility and the suppressing nature of the soil [44]. Krosin was responsible for inducing resistance in plants against *Ralstonia solanacearum*. Thus the integrated use of Krosin, Furadan, *Trichoderma* formulation, and Poultry manure showed the maximum performances in yield and yield contributing characters. Though treatment T₁₃ comprised of *Trichoderma* formulation and Poultry manure, it gave a lower yield than T₁₄. It might be due to the presence of component Autos-tin 50 WP which has antifungal properties on *Trichoderma* formulation.

4. Conclusions

In the current study, the morphological and cultural characteristics of *F. oxysporum*, *R. solanacearum* and *Meloidogyne* sp. have been described to provide key information for identification. The pathogenicity test confirmed the virulent nature of these pathogens which caused wilt of eggplant plants. Further, *in vitro* assay suggests that carbendazim can control *F. oxysporum* while streptomycin sulphate with tetracycline hydrochloride act against *R. solanacearum* effectively even at low concentrations.

From the field experiment, it can be concluded that integration of Streptomycin Sulphate with Tetracycline Hydrochloride (Krosin 10SP), Carbofuran (Furadan 3G), Poultry manure and *Trichoderma harzianum* formulation may be an alternative approach to control wilt complex of eggplant with higher economic return and lower impact on the environment. The feasibility of using this treatment in controlling wilt complex of eggplant needs to be tested both in other solanaceous vegetables irrespective of Agro-Ecological Zone (AEZ) of the country.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data availability Statement

The data that support the findings of this study are openly available in “Digital Archive on Agricultural Theses and Journal” at

http://www.saulibrary.edu.bd/daatj/public/index.php/thesis/thesis_individual/6596, reference number 11-04480_11.pdf.

References

- [1] BBS (2018) Year Book of Agricultural Statistics of Bangladesh. Statistics Division, Bangladesh Bureau of Statistics (Monthly Statistical Bulletin, Bangladesh, December, 2018), Ministry of Planning, Government of the Peoples' Republic of Bangladesh, Dhaka, Bangladesh, 65-67.
- [2] Das, G.P., Ramaswamy, S. and Bari, M.A. (2000) Integrated Crop Management Practices for the Control of the Eggplant Shoot and Fruit Borer in Bangladesh. DAE-DA NIDA Strengthening Plant Protection Services (SPPS) Project. Department of Agricultural Extension, Dhaka, 12.
- [3] Rashid, M.M. (2000) A Guidebook of Plant Pathology. Department of Plant Pathology, HSTU, Dinajpur, 58.
- [4] Ali, M., Alam, M.Z. and Akanda, M.A.M. (1994) Grafting: A Technique of Control Soil Borne Diseases of Tomato and Eggplant. Institute of Post Graduate Studies in Agriculture (IPSA), Gazipur, IPSA-JICA Publication No. 4, 10.
- [5] Vanitha, S.C., Niranjana, S.R., Mortensen, C.N. and Umesha, S. (2009) Bacterial Wilt of Tomato in Karnataka and Its Management by *Pseudomonas fluorescens*. *Biocontrol*, **54**, 685-695. <https://doi.org/10.1007/s10526-009-9217-x>
- [6] Nishat, S., Hamim, I., Khalil, M.I., Ali, M.A., Hossain, M.A., Meah, M.B. and Islam, M.R. (2015) Genetic Diversity of the Bacterial Wilt Pathogen *Ralstonia solanacearum* Using a RAPD Marker. *Comptes Rendus Biologies*, **338**, 757-767. <https://doi.org/10.1016/j.crv.2015.06.009>
- [7] Begum, H.A. (2007) Studies on the Integrated Management for Tomato Wilt Complex. PhD Thesis, Bangladesh Agricultural University, Mymensingh.
- [8] Meah, M.B. (1997) Diseases in Rabi Crops under Crop Diversification Program. Department of Agricultural Extension, Dhaka, Report Crop Diversification Program, 10.
- [9] Bari, M.A. (2001) Biological Control of Soil Borne Diseases of Vegetables. Contract Research Project, Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydevpur, 21-49.
- [10] Ka skavalci, G. (2007) Effect of Soil Solarization and Organic Amendment Treatments for Controlling *Meloidogyne incognita* in Tomato Cultivars in Western Anatolia. *Turkish Journal of Agriculture and Forestry*, **31**, 159-167.
- [11] Elphinstone, J.G. and Aley, P. (1993) Integrated Control of Bacterial Wilt of Potato in the Warm Tropics of Peru. *Bacterial Wilt Proceedings of an International Conference*, Kaohsiung, 28-31 October 1992, 276-283.
- [12] Kucharek, T. (1998) Bacterial Wilt of Row Crops in Florida. Cooperative Extension Service. Institute of Food and Agricultural Sciences, Gainesville.
- [13] Pradhanang, P.M., Momol, M.T., Olson, S.M. and Jones, J.B. (2003) Effects of Plant Essential Oils on *Ralstonia solanacearum* Population Density and Bacterial Wilt Incidence in Tomato. *Plant Disease*, **87**, 423-427. <https://doi.org/10.1094/PDIS.2003.87.4.423>
- [14] Chowdhury, M.A.Z., Fakhruddin, A.N.M., Islam, M.N., Moniruzzaman, M., Gan, S.H. and Alam, M.K. (2013) Detection of the Residues of Nineteen Pesticides in Fresh Vegetable Samples Using Gas Chromatography-Mass Spectrometry. *Food Control*, **34**, 457-465. <https://doi.org/10.1016/j.foodcont.2013.05.006>

- [15] Rahman, Z. (2016) Lack of Regulations Affect Vegetable Export. *The Financial Express*. <http://www.thefinancialexpress-bd.Com/2016/03/26/23070/asia/print>
- [16] Kulkarni, S. (2015) Commercialization of Microbial Bio Pesticides for the Management of Pests and Diseases. In: Awasthi, L.P., Ed., *Recent Advances in the Diagnosis and Management of Plant Diseases*, Springer, Berlin, 8. https://doi.org/10.1007/978-81-322-2571-3_1
- [17] Hermosa, R., Viterbo, A., Chet, I. and Monte, E. (2012) Plant-Beneficial Effects of Trichoderma and of Its Gene. *Microbiology*, **158**, 17-25. <https://doi.org/10.1099/mic.0.052274-0>
- [18] Howell, C.R. (2006) Understanding the Mechanism Employed by *Trichoderma virens* to Effect Biological Control of Cotton Diseases. *Phytopathology*, **96**, 178-180. <https://doi.org/10.1094/PHYTO-96-0178>
- [19] Ramaiah, A.K. and Garampalli, R.K.H. (2015) *In Vitro* Antifungal Activity of Some Plant Extracts against *Fusarium oxysporum* f. sp. *lycopersici*. *Asian Journal of Plant Science and Research*, **5**, 22-27.
- [20] Sarkar, S. and Chaudhuri, S. (2016) Bacterial Wilt and Its Management. *Current Science*, **110**, 1439-1445.
- [21] Janvier, C., Villeneuve, E., Alabouvette, C., Edel-Hermann, V., Mateille, T. and Steinberg, C. (2007) Soil Health through Soil Disease Suppression: Which Strategy from Descriptors to Indicators? *Soil Biology and Biochemistry*, **39**, 1-23. <https://doi.org/10.1016/j.soilbio.2006.07.001>
- [22] Islam, T.M.D. and Toyota, K. (2004) Suppression of Bacterial Wilt of Tomato by *Ralstonia solanacearum* by Incorporation of Composts in Soil and Possible Mechanism. *Microbes and Environment*, **19**, 53-60. <https://doi.org/10.1264/jsme2.2004.53>
- [23] Vyas, R.V., Patel, B.A., Patel, B.N. and Patel, J.G. (2009) Integrated Management of Root-Knot Nematode in Eggplant under Field Conditions. *Indian Journal of Nematology*, **39**, 35-37.
- [24] Kelman, A. (1954) The Relationship of Pathogenicity in *Pseudomonas solanacearum* to Colony Appearance on a Tetrazolium Medium. *Phytopathology*, **44**, 693-695.
- [25] Gram, H.C. (1884) Über die isolierte Färbung der Schizomyceten in Schnitt und Trockenpräparaten. *Fortschritte der Medizin*, **2**, 185-189. (In German)
- [26] Islam, M.M., Islam, M.R., Stevens, C., Meah, M.B. and Miah, M.A.T. (2005) Molecular Characterization of *Phomopsis vexans* in the Core Eggplant Producing Areas of Bangladesh. *Sixteen Biennial Conference of Bangladesh Phytopathological Society*, Dhaka, Bangladesh, 9 February 2016, 2.
- [27] Vincent, J.M. (1947) Distortion of Fungal Hyphae in Presence of Certain Inhibition. *Nature*, **159**, 850. <https://doi.org/10.1038/159850b0>
- [28] Islam, M.A., Sharfuddin, A.F.M. and Islam, N. (2004d) A Study of Production Technology and Disease Management of Ginger and Turmeric in Selected Areas of Bangladesh. *Bangladesh Journal of Crop Science*, **13**, 103-110.
- [29] Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. (1983) *Fusarium Species: An Illustrated Manual for Identification*. Pennsylvania State University Press, University Park.
- [30] Joshi, M., Srivastava, R., Sharma, A.K. and Prakash, A. (2013) Isolation and Characterization of *Fusarium oxysporum*, a Wilt Causing Fungus, for Its Pathogenic and Non-Pathogenic Nature in Tomato (*Solanum lycopersicum*). *Journal of Applied and Natural Science*, **5**, 108-117. <https://doi.org/10.31018/jans.v5i1.290>

- [31] Kumar, A. and Sarma, Y.R. (2004) Characterization of *Ralstonia solanacearum* Causing Bacterial Wilt in Ginger. *Indian Phytopathology*, **57**, 12-17.
- [32] Dhital, S.P., Thaveechai, N. and Shrestha, S.K. (2000/2001) Characteristics of *Ralstonia Solanacearum* Strains of Potato Wilt Disease from Nepal and Thailand. *Nepal Agriculture Research Journal*, **4-5**, 2000-2001.
- [33] Sitaramaiah, K. and Singh, R.S. (1976) Control of Root-Knot of Vegetable Crops. In: *Annual Report of Research 1974-75*, G.B. Pant University of Agriculture & Technology, Pantagar, 113-114.
- [34] Jahanshir, A. and Fevzi, S.D. (2010) The Effects of Fungicides on *Fusarium oxysporum* f. sp. *lycopersici* Associated with Fusarium Wilt of Tomato. *Journal of Plant Protection Research*, **50**, 172. <https://doi.org/10.2478/v10045-010-0029-x>
- [35] Murakoshi, S. and Takahashi, M. (1984) Trials of Some Control of Tomato wilt Caused by *Pseudomonas solanacearum*. *Bulletin of the Kanagawa Horticultural Experiment Station*, **31**, 50-56.
- [36] Milijasevic, S., Todorovic, B., Potocnik, I., Rekanovic, E. and Stepanovic, M. (2009) Effects of Copper-Based Compounds, Antibiotics and a Plant Activator on Population Sizes and Spread of *Clavibacter michiganensis* subsp. *michiganensis* in Greenhouse Tomato Seedling. *Pesticides and Phytomedicine*, **24**, 19-27. <https://doi.org/10.2298/PIF0901019M>
- [37] Adhikary, M.C., Begum, H.A. and Miah, M.B. (2017) Possibility of Recovery of Fusarium Wilt Affected Eggplants by Trichoderma. *International Journal of Agricultural Research, Innovation and Technology*, **7**, 38-42. <https://doi.org/10.3329/ijarit.v7i1.33319>
- [38] Pava, H.M., Franje, N.F. and Timario, T.J. (2003) Banana Pilot Demonstration Studies for Bukidnon: Table Salt and Early Debudding to Control “Bugtok” Disease of Cooking Banana Cultivars “Saba” and “Cardaba”. *Philippine Journal of Crop Science*, **28**, 31-43.
- [39] Zakir, H. and Bora, B.C. (2008) Integrated Management of *Meloidogyne incognita* and *Ralstonia solanacearum* Complex in Eggplant. *Indian Journal of Nematology*, **38**, 58-64.
- [40] Pavithra, S. and Khatib, R. (2014) Integrated Management of Wilt Complex Involving *Meloidogyne incognita* and *Ralstonia solanacearum* on Eggplant (*Solanum melongena* L.). *The Bioscan*, **9**, 291-294.
- [41] Harman, G.E. (2000) Myths and Dogmas of Biocontrol Changes in Perceptions Derived on *Trichoderma harzianum* T-22. *Plant Disease*, **84**, 377-393. <https://doi.org/10.1094/PDIS.2000.84.4.377>
- [42] Shores, M., Harman, G.E. and Mastouri, F. (2010) Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. *Annual Review of Phytopathology*, **48**, 21-43. <https://doi.org/10.1146/annurev-phyto-073009-114450>
- [43] Mahfouz, M.M., Elgawad, A.B.D., Sanaa and Kabeil, S.A. (2010) Management of the Root-Knot Nematode *Meloidogyne incognita* on Tomato in Egypt. *Journal of American Science*, **6**, 256-262.
- [44] Stirling, G.R. (1989) Organic Amendments for Control of Root-Knot Nematode on Ginger. *Australasian Plant Pathology*, **18**, 39-44. <https://doi.org/10.1071/APP9890039>