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Eco Friendly Management of Bacterial Wilt of Brinjal (*(Solanum melongena L*) for Sustainability and Livelihood of Small and Marginal Farmers of Assam

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ABSTRACT

The Brinjal (Solanum melongena L) is a common and popular vegetable crop grown extensively in Assam and major source of income for the small and marginal farmers of the state. The major constraint in the production of brinjal is the bacterial wilt disease caused by Ralstonia solanacearum. It constitutes a serious obstacle to the cultivation of brinjal, causing total damage of plantations before(15-23% loss) as well as after bearing fruits(54.6- 62.5%). Biological control through the use of natural antagonistic microorganisms has emerged as one of best eco friendly management system, a promising alternatives to chemical pesticide. Local strain of biocontrol agents were tested for finding out the best bio-agent in suppressing the attack of Ralstonia solanacearum on Brinjal under field condition. Among all tested combination, the treatment containing combination of Trichoderma viride + Pseudomonas fluorescens applied in Seed+ Root + Soil methods of application was found most effective in reducing the incidence(19.80%) of bacterial wilt disease under field situation. Highest per plant yield , (3866.77 g/plant) , plant height, fruit size(337.02 gram per fruit) and numbers were also recorded from the same combination and the same methods of application.

Key Words: Brinjal, Bacterial wilt, Bio control agents, Eco- friendly.

INTRODUCTION

Biological control through the use of natural antagonistic microorganisms has emerged as one of the promising alternatives to chemical control. Biological control appears to take place on the plant surface by the activity of epiphytic micro flora. This is an important consideration when applying chemicals to plants, since there is a risk of killing natural antagonists of pathogens other than the one being treated. The most common mechanisms for microbial antagonism of plant pathogens are parasitism, predation, competition, induced resistance and the production of antimicrobial substances. Often, several mechanisms act together. While it is unlikely that biological control will completely replace chemical pesticides in the foreseeable future, we can expect that there will be some decline in the use of chemicals. So far, most approaches have involved the single antagonist concept, although a biological systems approach, where disease is suppressed from several angles, might provide a better alternative. Similarly, the use of biological control agents could be used as one component of an integrated management program to achieve the best possible results.

The Brinjal *(Solanum melongena L*) is a common and popular vegetable crop grown extensively in Assam and is a major source of income for the small and marginal farmers of the state. The major constraint in the production of brinjal is the bacterial wilt disease caused by *Ralstonia solanacearum* (Yabuuchi *et al*, 1996). It is a soil borne and can survive in soil upto 1-10 years. It constitutes a serious obstacle to the cultivation of brinjal, causing total damage of plantations before as well as after bearing fruits. Bacterial wilt can account for 15 to 23% loss of brinjal crops before they bear any fruit and the average reduction in yield may be 54.6 to 62.5% due to further death of the bearing plants before full maturity(Das and Chattopadhyay,1955).(Chao *et al*,1997).Biological control could have an important role in the management of bacterial wilt (Akiew *et al*, 1993, Anuradha *et al.,* 1990). The specific objectives of the present study were to assess the nature and intensity of damaged caused by the bacterial wilt disease , study efficacy of different antagonist against the wilt disease under field condition. To evolve suitable Biocontrol measure and disease management strategies for growers.

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MATERIALS AND METHODS

Diseased leaf symptoms **of** Brinjal *(Solanum melongena L*) with suspected bacterial symptoms were collected for present study. The characteristics disease symptoms of the collected samples were observed and recorded. Leaf samples showing suspected bacterial were examined for bacterial ooze and bacterium were isolated from infected tissues in pure culture using streak plate method on Nutrient Agar Medium(NAM). Periodic transfer of the cultures were made to maintain the viability of the preserved bacteria. After isolation of the causal organisms the pathgenicity test was conducted through Koch's postulates (1882) by root inoculation technique (Winstead and Kleman,1952) . Another set of plants were inoculated with sterile distilled to serve as a control.

Bio agents used for management of bacterial wilt pathogen

Local strain of biocontrol agents like *Trichoderma viride, Trichoderma harzianum and Pseudomonas fluorescens* were collected and was tested in alone or combination with each other against *Ralstonia solanacearum* in field condition

Antagonists enriched Farm yard manure (*FYM*) for soil application:

One kg of talcum powder packets inoculated with bioagent like *Trichoderma viride*, *T. harzianum* and *Fluorescent Pseudomonas* each were mixed separately in 100 kg of FYM and sprinkled some amount of water over it to maintain the required humidity. After that the mixture was covered with gunny bags for 0 days. After 10 days of incubation the antagonist were rapidly multiplied in the farm yard manure and ultimately antagonist enriched FYM were produced which were ready for soil application.

Evaluation of potential biocontrol agents under field condition:

Local strain of biocontrol agents like *Trichoderma viride, Trichoderma harzianum* and *Fluorescens Pseudomonas* were tested either alone or in various combinations for finding out their efficacy in suppressing the *Ralstonia solanacearum* the causal agent of bacterial wilt diseases on Brinjal *(Solanum melongena L*) under field conditions.

Preparation of talc-based formulation of fungal and bacterial bio-agents

Culture of fungal cells were maintained on the potato Dextrose medium(PDA) and bacterial cell were maintained in nutrient agar (NA) medium. Both *Trichoderma spp*. and fluorescent *Pseudomonas* were multiplied separately in talcum powder at 1:3 ratios (v/w). Polypropylene bags with 1 kg finely sieved talcum powder were taken for multiplication of both fungi and bacteria, there added 10 ml filter water and exposed to steam in autoclave at 100 ^{0}C. 1% of Carboxy methyl cellulose (CMC) solution , 1% of Manitol solution and 0.1% of Humic acid solution were added to the Polypropylene bags containing 1 kg of talcum powder at the time of inoculation of antagonistic fungi or bacteria to hold it together. The mixture were incubated at 26 ± 2^0 for 24 hr for multiplication of *Pseudomonas fluorescent* and *Trichoderma spp* respectively. After 24 hr of incubation the packets were shacked manually for uniform spread of antagonist in the talcum powder. This operation was repeated after 48hr, 72 hr, 96hr, 120hr, 144hr of incubation, respectively. After 168 hr (7 days) of incubation and continuous shaking the inoculated packets were brought out from the incubator and evaluated for presence of required population of the antagonists (Vidhyasekaran and Muthamilan,1995).

Mass multiplication of antagonistic agents on Farm Yard Manure(FYM)

In order to get antagonist enriched FYM, 1kg of biopesticide was mixed with 100 kg of dry cow dung . The mixture was sprinkled with water and covered with gunny bags for its multiplication. After 10 – 12 days, the applied bioagent multiplied rapidly over the used dry cowdung and finally the 100kg of farm yard manure were converted into antagonist enriched FYM and ready for soil application.(Jeyarajan *et al*,1998; Jacob *et al*,1994). A field study was conducted at the with three replication during Oct-Feb for 3 consecutive years . Analysis of yield and disease incidence of bio agent treated crops: Data were recorded on Disease incidence (%), Yield/plant (g), Plant height, Number of fruits per plant, average fruit weight of the bio agent treated plants. Appropriate statistical tools were used to analyze the data following the procedure described (Gomez and Gomez, 1984).

Preparation of planting material/Collection of healthy seed and sowing in seed bed:

Healthy seeds of Brinjal were collected from the diseased free plot. In order to conduct field experiment of bio-agent efficacy against the wilt pathogen of Brinjal , seeds were sown in standard size of seed bed just after treated with antagonistic bacteria and fungi in alone or combination with each other @ of 1 gm antagonist / 10 gm of seeds . In order to protects the seed ling of both the crops from insect infestation, caging of nursery bed was done.

Treatment:

A1	*Trichodermaviride*
A2	*T. harzianum*
A3	*Pseudomonasfluorescens*
A4	*T. viride* + *P. fluorescens*
A5	*T. harzianum*+ *P. fluorescens*
A6	Chemicalcontrol
A7	Control

Seed treatment with antagonist (1 gm/ 10 gm of seed)
Root treatment with antagonist (1 kg in 2 litres of water for 1000 seedlings) &
Soil application with antagonist (100g antagonist enriched dried cow dung/plant)

Efficacy of antagonists on wilt disease incidence under field condition

In order to study the efficacy of bio control agents against the Bacterial wilt pathogen, field trials were conducted at the farmers sick plot during *Rabi* season for 3 consecutive years in 2018–19, 2019-20 & 2020-21. Brinjal cultivar Thur Bengena were grown in the nursery and transplanted into the field 3-4 weeks after sowing. Each plot consisted of 9 plants spaced 50 cm apart for brinjal . Treatments were arranged in Complete Block Design using Strip Plots with three replications. Chemical fungicide Copper oxychloride (0.1%) was used as the standard check. Treatments were assigned as described in the Materials and Methods.

Methods of application	
M1	Seed treatment
M2	Seed + Root treatment
M3	Seed + Soil treatment
M4	Seed +Root + Soil treatment

Number of Treatments 24					
A1M1	A2M1	A3M1	A4M1	A5M1	A6M1
A1M2	A2M2	A3M2	A4M2	A5M2	A6M2
A1M3	A2M3	A3M3	A4M3	A5M3	A6M3
A1M4	A2M4	A3M4	A4M4	A5M4	A6M4
Control					

Number of Replications: 3

Experiment Design: Complete Block Design using Strip Plots

RESULTS AND DISCUSSION

Ooze test for preliminary diagnosis of bacterial diseases:

White coloured slimy bacterial cells oozed out from all the suspected diseased samples Infected leaf sections also showed the oozing of bacterial cells, indicating that the disease is caused by bacteria. Samples without characteristics disease symptoms did not produce any ooze indicating healthy samples.

Isolation of the bacteria from the diseased plant parts:

The colonies were observed as Light pink in colour,opaque, circular, medium surface, entire margin with low convex elevation .

Pathogenicity Test:

Koch postulation of the isolate was performed to prove the pathogenicity by root inoculation technique. Inoculated seedlings of the plants

reproduced the typical symptoms observed on the naturally infected plant within 10 days. In control plants inoculated with sterile distilled water without bacterial inoculums, no such symptoms were reproduced.

The data (Table 1) represent the effect of tested treatments on yield and disease incidence in Brinjal under the field experiment. In field experiment, all the treatments were found significantly superior to the absolute control in reducing the incidence of the Bacterial wilt in Brinjal. The minimum disease incidence (24.43 %) was recorded from the combination of *T. viride* and *P. fluorescens* application among all antagonist treatments. However, this was the second best treatment when compared to Copper oxychloride (0.1%) application which reduced the disease incidence to 18.90 per cent. In case of absolute control the disease incidence was as high as 51.07 per cent. Among the individual antagonist *T. viride* was found the best recording 30.44 per cent disease incidence while *T. harzianum* was observed least effective showing the disease incidence of 41.62 per cent.

The highest average fruit yield (3896.93 g/plant) was recovered from chemically treated plants followed by combination of *T. viride* + *Pseudomonas fluorescens* with an average fruit yield of 3154.29 gram per plant. Among individual antagonist, *T. viride* performed the best to produced an average fruit yield of 2638.66 gram perplant. Plants treated with *Trichoderma harzianum* showed the lowest yield (1964.60 g/plant) among all the treatments. However, all the treatments gave statistically superior yield than the control.(Fig1)

Table 1. Effect of antagonist applied in different methods on yield and disease intensity of Brinjal under field condition

Antagonist	Yield(g/Plant)				Disease intensity (%)			
	Method of Application				Method of Application			
	M1	M2	M3	M4	M1	M2	M3	M4
T.viride (A1)	2054.987	2304.96	2681.76	3512.93	37.62	33.21	28.76	22.17
T. harzianum(A2)	1260.81	1513.13	1608.65	1862.33	45.16	41.66	40.35	39.32
Fluorescen Pseudomonas(A3)	1447.06	1682.32	1948.02	2241.36	41.77	40.44	36.94	34.47
*T.viride+P.fluorescens(*A4)	2605.58	2911.98	3232.83	3866.77	28.65	26.00	23.28	19.80
T.harzianum+ P.fluorescens(A5)	1624.91	2203.733	1429.12	2600.64	40.82	36.38	33.54	27.33
Chemical (A6)	3449.06	3504.97	4038.40	4595.29	20.33	20.78	18.15	16.32
Control(A7)	854.76	854.76	854.76	854.76	51.07	51.07	51.07	51.07

	CD (5%)	CV (%)	CD (5%)	CV (%)
Antagonists	205.55	7.08%	1.39	2.98%
Methods of application	173.42		1.18	
Antagonists x Methodof application	261.67		1.69	

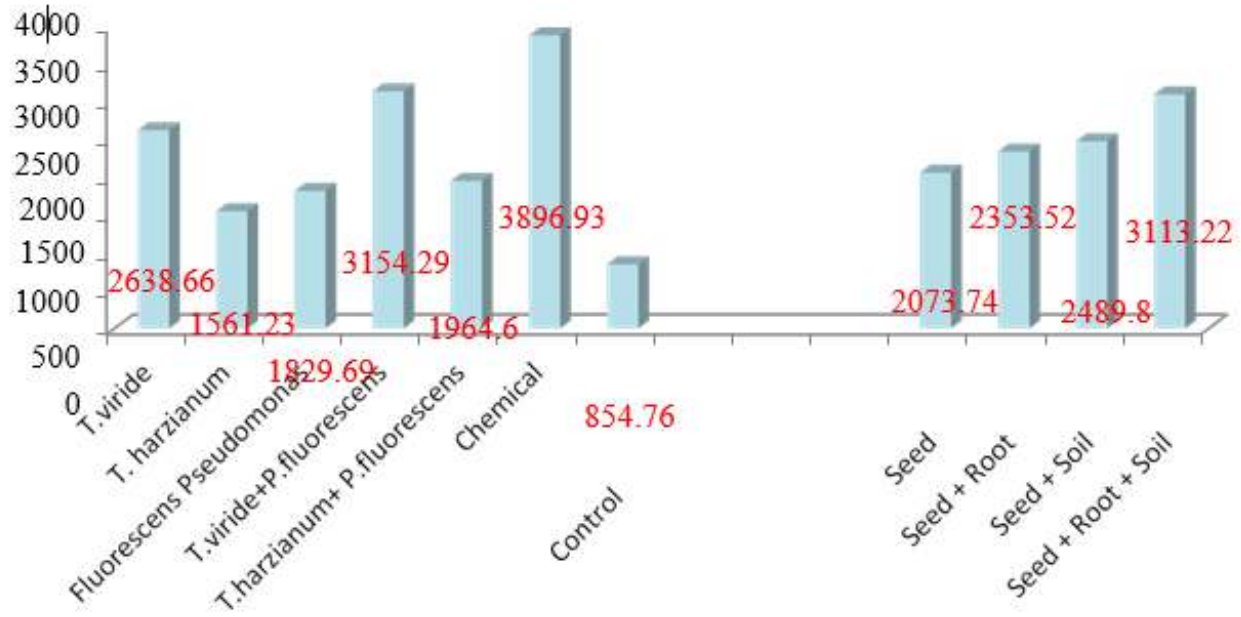

Fig.1. ***Average yield of Brinjal (g/plant) under different Antagonisttreatments and methods of application in field condition***

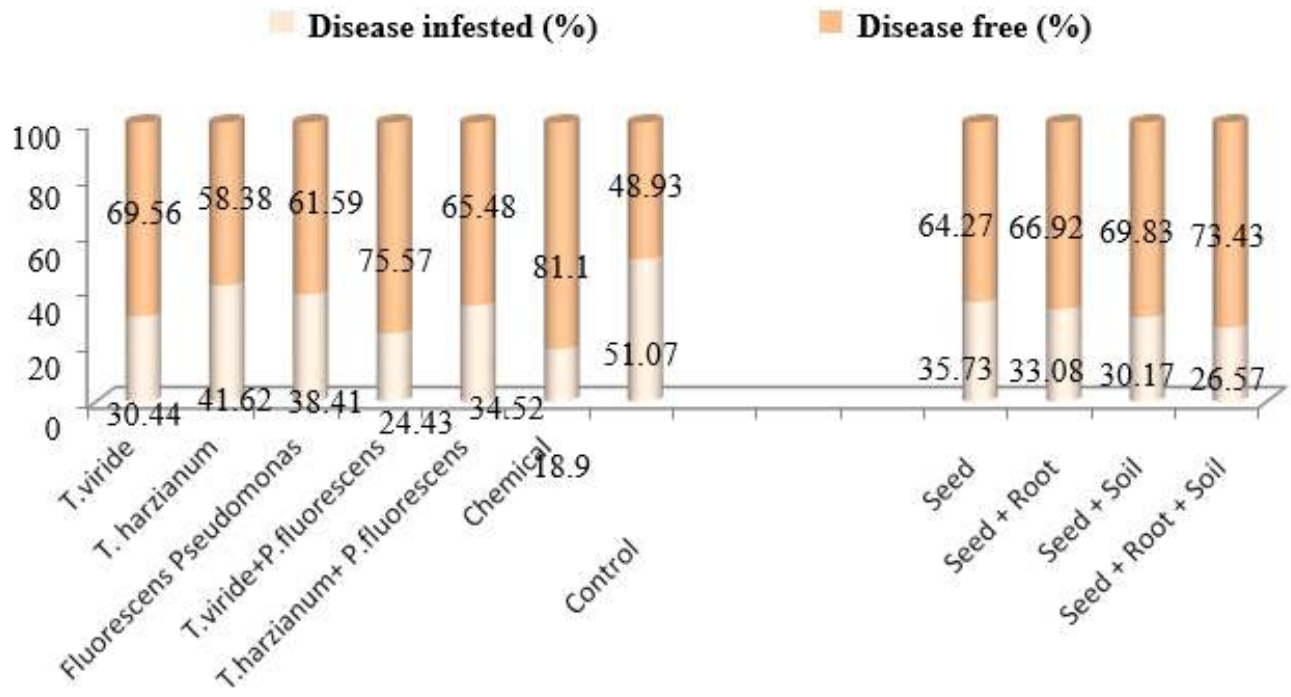

Fig.2*Average wilt incidence (%) of Brinjal under different Antagonist treatments and methods of application in field condition*

Comparison of different methods of application showed that the seed + root + soil method of application performed the best to minimise the disease incidence and to generate the maximum yield for all the antagonist and chemical treatments followed by seed + soil method of application.

Regarding interaction effect of antagonists/ chemicals and their methods of application,the data (Table1) show that treatment *T. viride* + *Pseudomonas fluorescens* applied through seed + root + soil method of application recorded the maximum yield (3866.77 g/plant) among all the tested treatments using antagonist and their combinations. However, the highest yield (4595.29 g/plant) among all the treatments was recorded from copper oxychloride treatment applied through seed + root + soil method of application.(Table1)

The data(Table 2) reveal that average plant height, average number of fruits and average fruit weight of Brinjal increased significantly under all the treatments comprising different antagonists and chemicals over the control. The highest average plant height (132.03 cm) was observed in copper oxychloride treated plants followed by *T.viride* + *Pseudomonas fluorescent* treatment (108.24) while highest average fruit weight of 337.02 gram per fruit was found in *T.viride* + *Pseudomonas fluorescent* treatment followed by copper oxychloride treated plants (334.88g/fruit). In respect of average number of fruits, *T.viride* + *Pseudomonas fluorescent* treatment was found at par with copper oxychloride treatment. Among the different methods of application, seed + root + soil method of application found the best to increase average plant height, average number of fruits and average fruit weight over all the treatments.

Table 2. Effect of antagonist in different methods on Plant height, fruit numbers and average fruit weight of Brinjal under field condition

Antagonist	Plant height(Cm)				Fruit numbers				Average fruit weight(g)			
	Method of Application				Method of Application				Method of Application			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
T.viride	90.57	102.92	105.79	112.70	8.00	8.33	9.33	10.00	256.87	276.31	297.97	351.29
T. harzianum	77.74	80.47	82.46	83.32	6.66	7.33	7.00	8.66	189.31	226.03	229.81	242.57
Fluorescens Pseudomonas	81.40	82.93	88.17	91.00	7.30	8.32	9.00	9.60	198.23	229.38	265.14	280.17
T.viride+ P.fluorescens	100.06	103.69	110.17	119.02	9.33	10.66	11.00	13.60	279.27	323.55	358.57	386.68
T.harzianum+ P.fluorescens	85.04	85.35	82.05	91.94	8.30	8.60	9.30	10.00	195.77	277.55	204.16	288.96
Chemical	130.35	129.67	132.61	135.48	10.3	11.00	11.60	13.6	334.86	318.63	348.14	337.89
Control	73.85	73.85	73.85	73.85	5.33	5.33	5.33	5.33	159.92	159.92	159.92	159.92

	CD (5%)	CV (%)	CD (5%)	CV (%)	CD (5%)	CV
(%)						
Antagonists	2.58	2.04%	0.36	7.54	11.94	3.41%
Methodsof application	1.07		0.782		7.91	
Antagonists x Methodof applications	3.08		1.22		14.87	

Lower incidence of Bacterial Wilt disease and higher fruit yield for the crops under study were recorded from *T. viride* + *P. fluorescens* treatment among all the antagonists and their combinations. This combination of *T. viride* + *P. fluorescens* performed the best showing the lowest disease incidence and the highest fruit yield in seed+ root + soil method of application under field condition. Maximum plant height, maximum number of fruits per plant and maximum fruit weight were also reported in same treatment and same method of application. Hence, it was evident from the present experimental results that the combination of *T. viride* + *P. fluorescens* is more effective against *Ralstonia solanacearum* causing Bacterial wilt disease in Brinjal compared to individual antagonist and the other combinations of antagonists considered under study. Rani *et al.,* (2006) also reported that combination of *T. harzianum* (TR20) + *P. fluorescens* (P28) was found most effective in reducing *Rhizoctonia . solani* in chilli. Manoranjitham *et al*., (2001) reported similar results for tomato. The result indicated the possibility of using of environment friendly native isolates of *Trichoderma viride* and *Pseudomonas fluorescens* based biopesticide for the management of bacterial wilt disease on Brinjal.

CONCLUSION

The results suggested that *Trichoderma viride* and *Pseudomonas fluorescens* based powder formulation of Biopesticide applied as seed treatment, root dip treatment and soil application is an excellent biological option to chemical methods for management of bacterial wilt of Brinjal , encouraging organic production. In addition to the positive impact of organic products on human health, this would provide the farmers a better market for their products as the demand for organic products have been increasing over time and space. Moreover, application of these antagonists further increase the yield , fruit numbers and fruit size of Brinjal ,which would enabling the farmers to earn more and thereby improving their economy and standard of living.

REFERENCE

Akiew E, Trevorrow PR, Tonells PE (1993). Management of bacterial wilt of tobacco. In: Bacterial wilt. Hartman GL and Hayward AC (eds.). ACIAR Proceedings. Australian Centre Int. *Agric Res Camera* **45**: 270-275.

Anuratha, CS., Gnanamanickam, SS. (1990**).** Biological control of bacterial wilt caused by *Pseudomonas solanacearum* in India with antagonistic bacteria. *Plant Soil* **124**: 109-116

Blair J E, Lenette E H and Truant J P (1971). In: *Laboratory Exercise in Microbiology* (Ed. Pleczer MJ, Chan EC). Mc. Graw Hill Book Co., Berlin, p. 356.

Bora L C and Deka S N (2007). Wilt disease suppression and yield enhancement in tomato (*Lycopersic esculentum*) by application of *Pseudomonas fluorescens* based biopesticide (Biofor-Pf) in Assam. *Indian J Agri Sci* **77**(8): 490-494.

Burr T J, Schroth M N and Suslow T (1978). Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *Pseudomonas putida. Phytopath* **68**: 1377-1383.

Das C R and ChattopadhyayS B (1955). Bacterial wilt of eggplant. *Indian Phytopath* **8**: 130-135.

Ganeshan G and Kumar MA (2006). *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *J Plant Interactions* **1**(3): 123-134.

Garrity M George (Eds.) (2001). Bergey's Manual of Systematic Bacteriology. Second Edition. Springer-Verlag, New York.

Gomez KA and Gomez AA (1984) *Statistical Productures for Agricultural Research*.2nded. Wiley, New York, 680pp.

Kelman A (1954). The bacterial wilt caused by *Pseudomonas solanacearum*. North Carolina Agric Exp Stn Tech Bull **99**: 194.

Kelman A and Person L H (1961) Strains of *P. solanacearum* differeing in pathogenicity to tobacco and peanut. *Phytopath* **51**: 158-161.

Manoranjitham S K, Prakasan V and Rajappan K(2001). Biocontrol of damping off of tomato caused by Pythium, aphanidermatum. *Indian Phytopath* **54**: 59-61.

Rani C R and Sulochana K K (2006). Management of seedling rot of chilli (Capsicum annum L.) using *Trichoderma spp.*and *fluorescent pseudomonas . J Tropical Agriculture* **44**:79-82.

Vidhyasekaran P and Muthamilan M (1995). Development of formulations o f *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis* **76**:782-786.

Winstead N N and Kelman A (1952). Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum. Phytopath* **42**: 628-634.

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