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Biological control of brinjal wilt caused by *Fusarium oxysporum* f. sp. *melongenae* using soluble powder formulation of *Aspergillus niger*

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Abstract

Fusarium wilt is the serious problem in brinjal. In the present study, *A. niger* has been isolated from the rhizosphere of brinjal, its soluble powder formulation was prepared using dextrose as a carrier, which has neutral pH, complete water solubility and long shelf life. Then bio-efficacy of the formulation was tested in a randomized block designed against *Fusarium oxysporum* f. sp. *melongenae* in pot trial through seed treatment. The results revealed that treatment "T5", dextrose base formulation of *A. niger*, at 10g per kg of seed showed maximum wilt disease control, (70.96%). This was followed by treatment "T6", thiram at 2g per Kg of seed and treatment "T1", talc-based *Trichoderma viride* at 10g per kg of seed showed (68.08%) and (64.53%) wilt disease control, respectively. Similarly, (87.33%) seed germination was recorded in treatment "T5" which was at par with treatment "T6" (90.0%) followed by treatment "T1" (83.92%). This work represented the antagonistic potential of *A. niger* against *Fusarium oxysporum* wilt of brinjal. Further evaluation of this isolate is necessary against other soil borne plant pathogens.

Keywords: *Solanum melongena*, *Aspergillus niger*, dextrose, *Fusarium oxysporum*, wilt control, germination

Introduction

Brinjal (*Solanum melongena* L.) is one of the most common, and highly productive vegetables grown globally and cultivated widely in India. Brinjal is susceptible to many diseases like *verticillium* wilt (*Verticillium dahliae*), *fusarium* wilt (*Fusarium oxysporum* f. sp. *melongenae*) and bacterial wilt (*Ralstonia solanacearum*) (Kalloo and Berg, 1993) and (Sihachakr *et al.*, 1994). *Fusarium* spp. is the most destructive pathogen that causes wilt in brinjal. This pathogen blocks the xylem transport system and causes severe wilting and death of brinjal plants (Altinok, 2005). Use of chemical, cultural and biological measures are some common practices followed to control this disease to some extent. Being a soilborne plant pathogen, it is difficult to control using a conventional chemical fungicides, because spores of this fungus survives for many years in the soil. Intensive use of chemical fungicides accumulates toxin in the environment and create residue problems. Rhizospheric microorganisms are the ideal control for soilborne plant pathogens. *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluorescens* are recommended for the control of soil borne plant pathogens.

In the present study, *Aspergillus niger* has been isolated and identified from the soil of brinjal field. Its soluble powder formulation was prepared using dextrose as a carrier and tested against the *Fusarium oxysporum* f. sp. *melongenae* of brinjal. *Aspergillus niger* is a haploid filamentous fungus, geographically widely distributed, found in wide range of habitat. *Aspergillus niger* has a wide range of industrial applications *viz:* production of extracellular enzymes like citric acids, amylases, lipases, cellulases, xylanases and proteases. It is also used for waste management and transformations. *Aspergillus niger* also causes black mold of onions and in certain fruits like grape, peanut, vegetable etc **(**Sharma Ruchi, 2012**).** In the present investigation, *A. niger* has been isolated from the soil of brinjal, multiplied on a solid substrate, harvested the spores and its soluble powder formulation was prepared using dextrose as a carrier having cfu count, 2 $x10^6$/g. After preparation of formulation, its viability, stability and bioefficacy were tested against *Fusarium oxysporum* which was isolated from infected roots of brinjal and the highest disease control was recorded at the dose of 10g per kg of seed through seed treatment which was significantly superior to chemical fungicide thiram as well as talc base market sample of *Trichoderma viride.*

Materials and Methods

A) Isolation of *Aspergillus niger* :

Soil sample collected from the infected field of brinjal, where wilting was observed. Ten-gram soil was taken in 3 replication and serially diluted with sterile distilled water from 10^{-1} to 10^{-4} dilutions and 100 µl of each dilution was plated onto Sabouraud dextrose agar plates and then incubated for 48 to 72 hrs at 28°C. After incubation, many types of fungal colonies were observed on plates. All fungal colonies were purified using single spore culture technique, then aggressively growing black colour fungus spores selected and purified. Fungi were further stained by lactophenol blue method and observed blue hyphae and fruiting structure of *A. niger*. Isolated *Aspergillus niger* grew on starch agar medium. After incubation, back mold appeared on plates with yellow zone around the colonies in the presence of iodine solution. Further, detailed morphological identification was done according to the colony colour, texture and margin along with microscopic characters like size of conidia, conidiophores and their arrangements as per the methods described by Raper & Fennell (1965).

B) Isolation of *Fusarium oxysporum* f. sp. *melongenae*:

Fusarium oxysporum was isolated from infected roots of brinjal plant. The infested portion of root part cut into small pieces and surface sterilized using 70% alcohol, followed by multiple washing with sterilized distilled water. Then washed pieces of roots were kept on PDA agar plate, that was prepared previously and plates further incubated at 25^0C for one week. After 8 days of incubation, *Fusarium* spp. was purified and identified using the microscopic charterers including the shape of conidia (Booth, 1977), primary and secondary characteristics according to the Nelson *et al*., (1983). After isolation and identification, a spore of *Fusarium oxysporum* inoculated in potato dextrose broth (PDB) and incubated up to 10 days at 120 RPM for inoculum preparation.

C) Strain revival and inoculum preparation:

Aspergillus niger which was isolated and identified was selected for the experiment. The culture was maintained on potato dextrose agar (PDA) slant at 28 ± 2^0 C. Using working slant, the fungal spore of *A. niger*, inoculated in potato dextrose broth upto 60 hrs at 28 ± 2^0 C on a rotary shaker for development of mycelium broth.

D) Mass multiplication of *Aspergillus niger*:

I) Solid state fermentation:

Solid state fermentation (SSF) is the cultivation of microorganisms under controlled conditions in the absence of free water. Mass multiplication of *A. niger* was carried out on substrate sorghum grains. Surface sterilization of substrate (sorghum grains) was made for the production of contamination-free conidia using chlorine water, followed by washing with RO water. After washing grains were filled in poly bags for autoclaving at 121^0C for 20 minutes.

II) Inoculation, incubation, and harvesting of conidia:

For production of conidia of *A. niger*, ten ml of sixty hrs old mycelium broth were inoculated into polybags separately, under laminar air flow chamber and incubated at 28^0C for 8 days for maximum conidia formation. Three replication were maintained. After sporulation, conidia were harvested aseptically from

sorghum grains, confirmed its purity, taken cfu count and then its water-soluble powder formulation prepared. Verified the spore count and stability of *A. niger* in dextrose formulation. Then bioefficacy of formulation was tested against *Fusarium oxysporum* of brinjal.

III) Bioefficacy testing:

Bioefficacy trial was conducted in two sets using seven different treatments to evaluate the results of a soluble powder formulation of *A. niger* against *Fusarium oxysporum* of brinjal. The inoculum of *Fusarium* prepared on rotary shaker, incorporated into the pots of sterilized soil in the proportion of 1:10 w/w. The pots were watered regularly to promote the fungal growth and kept for a month for uniform spreading of pathogen. Before conducting the trial, wilt incidence verified in the pots. Then *Aspergillus niger* formulation was used through seed treatment in different proportion (Table 1). First set of pot trial was used to record the observations of seed germination after 8 -10 days of sowing and for root length, shoot length, seedling vigour index (SVI) after 35 days of sowing and another set of trial was used to record the observations of wilt incidence and wilt disease control. Finally percent wilt incidence and percent wilt disease control were calculated. The brinjal seedling, as well as matured plants (foliage, flowers, fruits etc), were critically and frequently observed for the manifestation of the phytotoxic effects and abnormalities because of *A. niger* treatments.

Results

1. Isolation of *A. niger* and it's formulation development:

During our field visit of brinjal field, some aggressively growing black colour mold observed on one of the wilt affected part of brinjal. The soil sample of that infected areas was collected and transferred to the laboratory, isolated and identified the black colour fungus, which was *A. niger* and checked its antagonistic activities against *Fusarium oxysporum* which was further isolated from the infected roots of brinjal. In preliminary lab trials isolated fungus (*A. niger*) showed strong inhibition of *Fusarium oxysporum*. Therefore *A. niger*, sub-cultured in potato dextrose broth (PDB) and multiplied on sorghum grains for sporulation. After 8 days of incubation, spores were harvested and used for dextrose base powder formulation development. Aseptically harvested spores of *A. niger* mixed with carrier dextrose and achieve the cfu count 2 $x10^6$ spores per gram and used for bio efficacy testing against *Fusarium oxysporum*.

2. Effect of *A. niger* formulations on growth parameters:

The perusal of the data (Table 1) revealed that all the treatments exhibited significantly higher seed germination, root length, shoot length and seedling vigour index (SVI), over untreated control.

Table 1: Effect of dextrose based formulations of *A. niger* on seed germination, root length, shoot length and seedling vigour index (SVI) of brinjal

Tr. No.	Treatments	Seed Germination (%)	Root length(cm)	Shoot length (cm)	SVI
T1	Talc base *T. viride* 10g/kg seed (Control)	83.92 (55.43)	4.75	10.00	1214.14
T2	Dextrose base *A. niger* 1g/kg seed	63.33 (39.30)	3.85	6.95	684.09
T3	Dextrose base *A. niger* 3g/kg seed	68.00 (42.85)	4.06	7.60	792.44
T4	Dextrose base *A. niger* 5g/kg seed	72.67 (46.61)	4.57	8.66	961.24
T5	Dextrose base *A. niger* 10 g/kg seed	87.33 (60.86)	6.02	12.55	1621.95
T6	Thiram 2g/kg seed	90.00 (64.23)	4.99	10.91	1430.36
T7	Control (Untreated)	43.33 (25.04)	2.97	5.73	668.46
	SE	0.93	0.39	0.12	18.60
	CD (P=0.05)	2.87	1.20	0.38	57.22

However, seed treatment "T6" with thiram at 2g per Kg of seed recorded significantly highest seed germination (90.00%) which was at par with the treatment "T5" of soluble powder formulation of *A. niger* at 10g per Kg of seed (87.33%), followed by treatment "T1" with talc based formulation of *Trichoderma viride* at 10g per Kg of seed (83.92%), whereas treatment "T5" also provided significantly highest root length (6.02cm), shoot length (12.55cm) and SVI (1621.95), followed by the treatment "T6" (4.99 cm, 10.91cm and 1430.36) and treatment "T1" (4.75 cm, 10.00cm and 1214.14) with root length, shoot length and SVI, respectively. Rest of the treatments also recorded improved seed germination (63.33 to 72.67%), root length (3.85 to 4.57cm), shoot length (6.95 to 8.66 cm) and SVI (684.09 to 961.25) as against significantly least seed germination (43.33%), root length (2.97 cm), shoot length (5.73cm) and SVI (668.46) in untreated control.

3. Wilt disease incidence and wilt disease control:

The data (Table 2) revealed that all the treatments recorded significantly minimum wilt incidence and maximum disease control as compared to untreated control. However, the treatment "T5" with dextrose base *A. niger* at 10g per kg of seed recorded significantly least wilt incidence (18.23%) and highest wilt disease control (70.96%). This was followed by treatment "T6" with thiram at 2g per kg of seed (20.04%, 68.08%) and treatment "T1" with talc-based *Trichoderma viride* at 10g per kg of seed (22.27%, 64.53%) with wilt incidence and wilt disease control respectively. Rest of the treatments also recorded comparatively minimum wilt incidence (34.77 to 36.25%) and maximum wilt control (42.28 to 44.63%) as against significantly highest wilt incidence (62.79) in untreated control.

Table 2: Effect of *A. niger* formulations on wilt incidence and wilt disease control of brinjal

Tr. No.	Treatments	Disease Incidence (%)	Disease Control (%)
T1	Talc base *T. viride* 10g/kg seed (Control)	22.27 (12.86)	64.53 (40.18)
T2	Dextrose base *A. niger* 1g/kg seed	36.25 (21.25)	42.28 (25.00)
T3	Dextrose base *A. niger* 3g/kg seed	35.95 (21.06)	42.75 (25.31
T4	Dextrose base *A. niger* 5g/kg seed	34.77 (20.34)	44.63 (26.50)
T5	Dextrose base *A. niger* 10 g/kg seed	18.23 (10.50)	70.96 (45.20)
T6	Thiram 2g/kg seed	20.04 (11.56)	68.08 (42.90)
T7	Control (Untreated)	62.79 (38.89)	-
	SE	0.10	0.16
	CD (P=0.05)	0.33	0.49

4. Phytotoxicity:

Dextrose base formulation of *A. niger* which was used for seed treatment of brinjal at various concentration also studied for phytotoxic effects on brinjal crop. However, *A. niger* formulation did not express any phytotoxic effect or abnormalities on brinjal crop. No epinasty, hyponasty, necrosis, chlorosis or any other leaf injury were observed on leaves of the brinjal plants in any of the treatment.

Discussion

Brinjal is an important vegetable crop grown worldwide which attacked by several diseases of fungi and bacteria. Among them, *Fusarium* wilt is an important constraint in brinjal production because *Fusarium* spp. penetrate through the roots and proliferate in vascular tissue (Boyaci *et al.*, 2011) which makes it difficult to control. In the present attempt, black colour mold *A. niger* was isolated from brinjal field. After primary evaluation, its water-soluble powder formulation was prepared using

dextrose as a carrier and tested against *Fusarium oxysporum* f. sp. *melongenae* of brinjal. *Aspergillus niger* is one of the species which has tremendous biotechnological applications and it has "GRAS status" (Generally Regarded As Safe) by the "Food and Drug Administration". However, some species reported as a plant disease-causing agents. According to Ruchi Sharma, (2012), *A. niger* causes black mold in onion, grapes, vegetable etc. But in the present study, isolated strain of *A. niger* showed strong antagonistic activity against *Fusarium oxysporum* f. sp. *melongenae* and control the wilt, significantly.

Along with the *Fusarium* wilt control, various growth parameter of brinjal also recorded. The data (Table 1) revealed that dextrose base formulation of *A. niger* was tested through seed treatment at various doses like 1g to 10 g per kg of seed of brinjal. Chemical fungicide thiram and talc base market samples of *Trichoderma viride* were used as a control. Dextrose base formulation of *A. niger* at 10g per kg of seed showed the highest seed germination. Not only seed germination of brinjal improved by the formulations but maximum root and shoot length along with maximum seed vigour index also recorded and which was statistically significant as compared to rest of the treatments. Role of *A. niger* AN 27 was reported for the rhizospheric competence and plant growth promotion (Loper and Buyer, 1991). The results obtained in the present investigation also indicated that isolated strain of *A. niger* might have produced plant growth promoting molecules in the rhizosphere soil and because of that seed germination improved along with the highest shoot and root length.

Microorganisms that can grow in the rhizosphere are ideal biocontrol agents. Talc based market formulation used as a control in the present experiment because various strains of *Trichoderma spp.* recommended for the control of *Fusarium* wilt in many crops (Morsy EM *et al.*, 2009) and (Sabalpara AN, 2009). *Trichoderma viride* produces various enzymes like chitinases, proteases and glucanases which decomposed the cell wall of phytopathogens. Chitinase produced by *Trichoderma viride* has fungicidal effects (Bell DK *et al.*, 1982). *Aspergillus niger* distributed widely and survives at a wide range of temperature. After application, fungal spores remains in the soil for long period of time. Some species of *Aspergillus* reported inhibitory against several plant pathogens (Getha *et al.*, 2005). *Aspergillus niger* AN27 also reported for the production of hydroxamate and catecholate type of siderophores and siderophore having a role in antagonistic activities (Loper and Buyer, 1991). According to the Maria SB & Urszula J (2012) *Aspergillus niger* LOCK 62 produce an anti fungal chitinases enzymes.

In the present study, it was observed that formulation of isolated *Aspergillus niger* control *Fusarium* wilt significantly. *Aspergillus niger* is a fast growing and very aggressive fungus, which might have created competition for nutrients and space with *Fusarium* and ultimately *Fusarium* incidence reduced. *A. niger* also reported for the control of tomato wilt causing agent *Fusarium oxysporum* f. sp. *lycopersici.* (Sharma BK *et al.*, 2011). The bio-control activity of *A. niger* formulation reported in the present study might be either through directly antagonism or production of secondary metabolites including iron chelating siderophores, antibiotics, lytic enzymes and toxins. Formulation of *Aspergillus niger* also tested for phytotoxicity and no phytotoxic symptoms were recorded in any of the treatment. The present study demonstrated that dextrose base formulation of isolated *Aspergillus niger* have potential to control brinjal wilt caused by *Fusarium oxysporum* f. sp. *melongenae.*

Dextrose sugar having many advantages when used as a carrier for bioagent like complete water solubility, formulations are most suitable for drenching and spraying applications, dextrose may act as a source of carbohydrate for inhabitant microbes of soil. This formulation is most convenient to the users for drip irrigation and spraying applications. According to the earlier observations made by Patil VM *et al.*, (2017), when dextrose sugar used as a carrier for *Bacillus subtilis* then seed germination increases and *Fusarium* wilt of castor controlled significantly, as compared to talc base formulation. The results reported in the present study are in agreement with those reported by Patil VM *et al.*, (2017).

Conclusion

It could be concluded that application of dextrose base formulation of *A. niger* increases the seed germination percentage, root and shoot length of brinjal crop along with the significant reduction of *Fusarium* wilt of brinjal. Dextrose base bio-control formulation of isolated strain of *Aspergillus niger* will be the best biological control against *Fusarium* spp with longer shelf life. It is not only cost-effective but eco-friendly and suitable for organic farming, export-oriented production of fruits, vegetables, flower etc. Further evaluation of this strain of *A. niger* is needed along with the efficacy testing against various soilborne plant pathogens of multiple crops.

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