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RESEARCH ARTICLE

ANTAGONISTIC EFFECT OF LIQUID BIO FORMULATIONS AGAINST WILT OF COTTON CAUSED BY *FUSARIUM OXYSPORUM* F.SP. *VASINFECTUM*

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ABSTRACT

A pot trial study was conducted to determine the efficacy of combined application of both fungal and bacterial antagonistic organism against *Fusarium* wilt of cotton. Further, also to estimate the rhizosphere population of antagonistic organism. Among the treatment, combined application of *T. viride* + *P. fluorescens* @10ml kg^{-1} and SA @2.5lit ha^{-1} (T_3) recorded minimum disease incidence (15.57 %) which was on par with the fungicide (14.20%) and also recorded maximum shoot length, root length and plant biomass over untreated control recorded maximum disease incidence (60.86%) and minimum growth parameters. With regard to estimation of rhizosphere population T_3 recorded minimum population of *Fusarium oxysporum* f.sp. *vasinfectum* (10.30×10^{-6}) and maximum rhizosphere population of 25.20×10^{-3} cfu g^{-1} soil of *T.viride* (Tv_3) and 30.10×10^{-6} cfu g^{-1} soil of *P. fluorescens* (Pf_7).

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INTRODUCTION

Cotton (*Gossypium* spp.) regarded as "white gold" (Shah *et al.,* 2011; Akhtar *et al.,* 2013) is one of the important and oldest commercial crops, plays a key role in the economic and social affairs of the world. China ranks first in the world in cotton production with 33 million bales, followed by India (25 million bales), and the United States (16.7 million bales) (USDA, 2010). In Tamil Nadu cotton is cultivated in 1.17 million ha during 2013-2014 with production of 2.80 million bales of lint and productivity (lint) of 726 kg/ha (Cotcorp.gov.in., 2014). In India, the productivity of cotton is very low due to many constraints including diseases. Cotton is affected by various diseases caused by fungi, bacteria and viruses. Of these diseases, wilt of cotton (*Gossypium* spp.), a vascular disease caused by the soilborne pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Snyder and Hans is an important pathogen, distributed worldwide. Management of *F. oxysporum* f.sp. *vasinfectum* using chemical fungicides has been the prevailing control method for over fifty years. Though effective fungicides are available to manage the soil borne diseases, they will not be reliable as a long term solution because of the concerns about exposure risks, health and environment hazards.

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Moreover, the frequent applications of fungicides may lead to the development of tolerance in the target organisms. As a result, in recent years, the biological control especially using fungal and bacterial antagonists against fungal plant pathogen has gained considerable attention and appears to be promising as a viable alternative to chemical control (Papavizas, 1985: Howell *et al.,* 1987). Management of soil borne diseases through biological control by addition of antagonistic microorganisms is known to be ecofriendly and effective method (Cook and Baker, 1983). Members of the genus *Pseudomonas* and *Trichoderma* have been known for their potential antifungal, plant growth promoting and plant defense inducing activities (Zaidi *et al*., 2004). In this direction the present investigation was carried to study the individual and combination effect of using an effective biocontrol agent for the sustainable eco-friendly management of wilt disease of cotton.

MATERIALS AND METHODS

Isolation of *Fusarium oxysporum* f.sp. *vasinfectum*

The pathogen *F. oxysporum* f.sp. *vasinfectum* was isolated from the diseased roots of cotton plants showing the typical wilt symptoms by tissue segment method (Rangaswami, 1972). Infected roots and stems were washed in tap water and

cut into small pieces. The pieces were surface sterilized in 1 per cent sodium hypochlorite ($NaOCl_2$) solution for 30 sec. and washed serially in sterile distilled water to remove the traces of sodium hypochlorite and then transferred to sterilized Petri plate containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature (28±2°C) for 5-7 days. Hyphal tips growing from infected bits were transferred to PDA slants and the fungus was purified by using hyphal tip technique (Rangaswami, 1972) and were preserved in a refrigerator at 4°C and used for further studies. The pathogen *F. oxysporum* f.sp. *vasinfectum* was identified with the help of the descriptions by Booth (1971) and Singh (1987). The pathogenicity of the isolates was proved by Koch's postulates.

Mass multiplication of *F. oxysporum* f.sp. *vasinfectum* inoculum for soil application

The isolates of the pathogen were multiplied in sand maize medium (Riker and Riker, 1936). Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content, filled in 500ml conical flask and autoclaved at 20 psi for two h. Four actively growing mycelial discs (9 mm) of the pathogen isolates were inoculated into each flask under aseptic condition and the flasks were incubated at room temp. (28+2°C) for 15 days the inoculum thus obtained was used for the experiments.

Isolation of native antagonists from rhizosphere soil

***Trichoderma* spp.**

Cotton rhizosphere soil samples collected from different locations were used for the isolation of *Trichoderma* isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). These strains of *Trichoderma* spp. were, purified following single hyphal tip method and maintained in TSM slants at 4^0C in refrigerator with periodical sub-culturing. *Trichoderma* spp., thus isolated was subjected for identification based on the key to species suggested by Domsch *et al*. (1980).

***Pseudomonas* spp.**

Pseudomonas spp. were isolated from the rhizosphere soil collected during the survey. The soil along with root bits was mixed thoroughly and one g of rhizosphere soil was processed following serial dilution. One ml of 10^{-5} dilution was plated on King's B (KB) agar medium and incubated at room temperature (28 ± 2^0C) for 48 hours (Aneja, 2003) to isolate *Pseudomonas*. The colonies fluorescencing under UV light were picked up, purified and maintained in KB slants. The efficient *Pseudomonas* strains identified from the *in vitro* dual culture assay were examined for the colony morphology, growth, pigmentation, cell shape and gram reaction as per the standard procedures given by Barthalomew and Mittewer (1950).

Preparation of liquid formulation of biocontrol agents

For the preparation of liquid formulations the method suggested by Manikandan *et al*. (2010) was followed. The most effective isolate of *P. fluorescens* and *T. viride* identified in the present study was multiplied on King's B and PDA broth respectively. The mother culture of *T. viride* and log phase culture of *P. fluorescens* was inoculated individually into broth and incubated at room temperature (28 ± 2°C). Further, the broth was added with glycerol at 2 per cent level. After the incubation period, the formulation was assessed for adequate CFU (>1×10^{-9} cfu ml^{-1}) following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for further studies.

Efficacy of seed treatment plus soil application with the antagonists on plant growth and wilt incidence of cotton (pot culture)

Sterilized soil was mixed with the pathogen inoculum @ 5 per cent (w/w) level and filled in 30 cm earthen pots. The antagonists meant for soil application were applied to the pots and incorporated well. Surface sterilized cotton seeds were treated with the antagonists as per the schedule. Surface sterilized cotton seeds sown in pot soil mixed with the inoculum of *F. oxysporum* f.sp. *vasinfectum* alone served as control. Seed treatment @ 4 g kg^{-1} of seed plus soil drench @ 0.1% with Carbendazim was used for comparison. The experiment was conducted with three replications per treatment in a randomized block design and five pots per replication were maintained with one plant maintained in each pot. All the observations *viz.,* plant growth parameters, wilt incidence, the population of the antagonists and pathogen in the rhizosphere was estimated at harvest using suitable selective media and serial dilution technique.

RESULTS AND DISCUSSION

Efficacy of seed treatment plus soil application of the antagonists against wilt incidence and plant growth of cotton (Pot culture)

The results obtained on the efficacy of combined delivery system of the antagonists *viz.,* seed plus soil treatment are furnished in Table 1. Among the antagonists tested by seed treatment + soil application, combination treatment (T_3) of *T. viride* and *P. fluorescens* recorded the minimum incidence of wilt with (15.57%) which was on par with the fungicide treatment (14.20%) and also recorded the maximum shoot length (68.56 cm), root length (29.50 cm) and plant biomass (150.34 g). Carbendazim as seed treatment @ 4g kg^{-1} of seed plus soil drenching @ 0.1% recorded 64.45 cm of shoot length, 27.45 cm of root length and 99.24 g of plant biomass. Individual application of *T. viride* and *P. fluorescens* as seed and soil treatment recorded a disease incidence of 20.90 and 17.80 per cent respectively. The untreated control recorded the maximum disease (60.86%) incidence and minimum growth parameters of cotton.

Seed treatment plus soil application of *T. viride* and *P. fluorescens* in combination resulted in significantly the lowest root rot incidence of blackgram (Sethuraman *et al*., 2003). Seed and soil application with the mixture of *T. viride* and *P. fluorescens* was more effective in reducing the root rot incidence and increasing seed germination of blackgram (Sajeena *et al*., 2004). The combination of delivery systems

might have supported better rhizosphere competence of the antagonists and the various mechanisms elicited by the biocontrol agents might have suppressed the pathogen resulting in the reduced incidence of the disease.

rhizosphere population of both the antagonist might be attributed to the reason that the co inoculated strains might not have influenced each other *in vivo* by spatial separation on the roots or the production of the inhibiting secondary compounds as observed by Duffy *et al*. (1996).

Table 1. Effect of Seed treatment and soil application with antagonistic organism on plant growth promotion and percent wilt incidence of Fusarium wilt of cotton (Pot trial)

T. No.	Treatments	Shoot length (cm)	Root length (cm)	Bio mass (g plant^{-1})	Per cent wilt incidence	Per cent decrease over control
T_1	*T. viride* ST @10 ml kg $^{-1}$ and SA @ 2.5 lit ha^{-1}	63.86	23.85	144.16	20.90	65.65
T_2	*P. fluorescens* ST @10 ml kg $^{-1}$ and SA @ 2.5 lit ha^{-1}	65.33	26.75	146.81	17.80	70.75
T_3	*T. viride* + *P. fluorescens* ST @ 10 ml kg $^{-1}$ and SA @ 2.5 lit ha^{-1}	68.56	29.50	150.34	15.57	74.12
T_4	Carbendazim 50% WP ST @ 4.0 g kg^{-1} and SA @ 0.1%	64.45	27.45	99.24	14.20	76.66
T_5	Control	45.60	20.16	60.46	60.86	–

Table 2. Effect of seed treatment and soil application with antagonistic organism on the rhizosphere population of cotton (Pot trial)

T. No.	Treatments	Rhizosphere population (g^{-1} of oven dry soil)		
		T. viride (10^3cfu)	*P. fluorescens* (10^6cfu)	FoV (10^3cfu)
T_1	*T. viride* ST @10 ml kg $^{-1}$ and SA @ 2.5 lit ha^{-1}	35.88	0.00	15.70
T_2	*P. fluorescens* ST @10 ml kg $^{-1}$ and SA @ 2.5 lit ha^{-1}	0.00	40.10	12.50
T_3	*T. viride* + *P. fluorescens* ST @ 10 ml kg $^{-1}$ and SA @ 2.5 lit ha^{-1}	25.20	30.10	10.30
T_4	Carbendazim 50% WP ST @ 4.0 g kg^{-1} and SA @ 0.1%	0.00	0.00	09.55
T_5	Control	0.00	0.00	27.66

Also the growth promoting substances produced by the combination of antagonists might have increased the growth parameters of cotton. Enhanced disease suppression with combination of delivery systems (seed and soil application) of individual antagonists was also observed by several workers in pigeon pea (Prasad *et al.,* 2002), in grams (Subramanian *et al.,* 2003), in maize (Meena *et al*., 2003), in rice (Lakshmi Tewari and Rajbir Singh, 2005), in castor (Raoof *et al.,* 2006) and in chick pea (Poornima Sharma, 2011). Sandheep *et al.,* (2012) reported that using of *T. harzianum* with *P. fluorescens* through soil mixing plus root dipping treatment could be provided not only additional protection against crop loss due to *Fusarium* diseases but also significantly increased vegetative growth of vanilla.

Effect of seed plus soil application with antagonists on the rhizosphere population of the bio agents and *F. oxysporum* f.sp. *vasinfectum* (Pot culture)

Seed treatment plus soil application with combination of antagonists *T. viride* + *P. fluorescens* recorded the minimum population of *F. oxysporum* f.sp. *vasinfectum* (10.30×10^{-6}) and recorded a rhizosphere population of 25.20×10^{-3} cfu g^{-1} soil of *T. viride* (Tv_3) and 30.10×10^{-6} cfu g^{-1} soil of *P. fluorescens* (Pf_7). The combined delivery system with *T. viride* alone reduced the pathogen population to 15.70×10^{-3} cfu g^{-1} soil and recorded a rhizosphere population of 35.88×10^{-3} cfu g^{-1} soil. Similarly, the combined delivery system with*P. fluorescens* (Pf_7) alone recorded a rhizosphere population of 40.10×10^{-6} cfu g^{-1} soil and reduced the pathogen population to 12.50×10^{-6} cfu g^{-1} soil. Seed treatment (4 g kg^{-1}) and soil drenching (0.1%) of Carbendazim caused the maximum reduction of the rhizosphere population of *F. oxysporum* f.sp. *vasinfectum* (9.55×10^{-3} cfu g^{-1}) as against 27.66×10^{-3} cfu g^{-1} soil in control (Table 2). The increase in the

Four or five fold increase in the population of *Trichoderma* spp. due to seed treatment was found by Ahmad and Baker (1987). *Trichoderma* spp. were reported as effective rhizosphere colonizers and were typically present at higher populations at the upper portion of the root, less at the central portion and again at increased population near the root tip (Sivan and Chet, 1989). Similarly, *P. fluorescens* was reported to posses more capacity to adhere to the plant root (Van Peer *et al.,* 1990) and the production of fili (finbriae) was quoted responsible for this (Vesper, 1987).

Anderson *et al.* (1988) reported that the ability of *Pseudomonas* to attach to a particular plant glycoprotein (agglutin) correlated to its colonization potential. The reduction in the population of the pathogen might be attributed to the direct action of the antagonists and the action of the metabolites produced by the bio agents, which might have suppressed the pathogen. The mechanisms by which the antagonists act upon pathogens include antibiotic production, competitive ability, direct parasitism and lysis (Raaijmakers *et al*., 1997). When *T. viride* and *P. fluorescens* were applied together as seed and soil application highest rhizosphere inoculum densities of the antagonists and lowest *M. phaseolina* inoculum density were observed by Sajeena *et al*. (2004). Thus, our studies have proved that application of *T. viride* and *P. fluorescens* in combination using combined delivery systems could be used successfully for the effective and sustainable management of cotton wilt disease.

REFERENCES

Ahmad, J.S. and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. *Phytopathology*, 77: 182-189.

Akhtar, K.P., R. Ullah, I.A. Khan, M. Saeed, N. Sarwar and S. Mansoor, 2013. First Symptomatic Evidence of Infection

of *Gossypium arboreum* with Cotton Leaf Curl Burewala Virus through Grafting. *Int. J. Agric. Biol.,* 15: 157–160.

Anderson, A.J., Tari, P.H and Tepper, C.S. 1988. Molecular studies on the role of a root surface agglutin in adherence and colonization by *Pseudomonas putida*. *Appl. Environ. Microbiol.,* 54: 375-380.

Aneja, K.R. 2003. *Experiments in Microbiology Plant Pathology and Biotechnology,* 4th ed., New Age International Publishers, New Delhi.

Barthlomew, J. W and Mitew r, T. 1950, A simpled bacterial staining. *StainTecholgy,* 25:13.

Booth, C. 1971. The Genus Fusarium. Common Wealth Mycological Institute, Kew, Surrey, England. pp. 237.

Cook, R.J. and Baker, K.F. 1983. The nature and practice of biological control of plant pathogens. The American Phytopathological Society, St. Paul, MN. P. 539.

Cotcorp.gov.in., 2014, cotcorp.gov.in.

Domsch, K.H., Gans, W. and Anderon, T.H. 1980. Compendium of soil fungi. Academic Press Ltd., London. p. 859.

Duffy, B.K., Simon, A. and Weller, D.M. 1996. Combination of *Trichoderma koningii* with fluorescent *Pseudomonads* for control of Take-all on wheat. *Phytopathology,* 86: 188-194.

Elad, Y. and Chet, I. 1983. Improved selective media for isolation of *Trichoderma* or *Fusarium* spp. *Phytoparasitica,* 11: 55-58.

Howell, C, R. 1987. Relevance of mycoparasitism in the biological control of *Rhizoctonia solani* by *Gliocladium virens* . *Phytopathology*., 77: 992-994.

Lakshmi Tewari and Rajbir Singh. 2005. Biological control of sheath blight of rice by *Trichoderma harzianum* using different delivery systems. *Indian Phytopath*., 58(1): 35-40.

Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T. and Samiyappan, R. 2010. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against *Fusarium* wilt of tomato. *Biol. Control,* 54: 83-89.

Papavizas, G.C. 1985. Survival of *Trichoderma* and *Gliocladium*: Biology, Ecology and potential for biocontrol. *Annu. Rev. Phytopathol*., 18: 389-413.

Poornima Sharma, 2011. Evaluation of disease control and plant growth promotion potential of biocontrol agents on and comparison of their activity with popular chemical control agent carbendazim. *Journal of Toxicology and Environmental Health Sciences*, 3(5):127- 138.

Prasad, R.D., Rangeeswaran, R., Hegde, S.V. and Anuroop, C.P. 2002. Effect of soil and seed application of *Trichoderma harzianum* on pigeonpea wilt caused by *Fusarium udum* under field conditions. *Crop Prot.,* 21: 293-297.

Raaijmakers, J.M., Weller, D.M. and Thomashow, L.S. 1997. Frequency of antibiotic producing *Pseudomonas* spp. in natural environments. *Appl. Environ. Microbiol*., 63: 881-887.

Rangaswami, G. 1972. Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, pp. 520.

Raoof, M.A., Ramabhadra, R. and Mehtab, Y. 2006. Bio control potential and shelf life of *Trichoderma viride* for the management of castor wilt. *Indian J. Pl. Prot.,* 34(1): 75-80.

Riker, A.J. and Riker, A.S. 1936. Introduction to research on plant diseases. John. S. Swift, C.M.C., New York. p. 117.

Sajeena, A., Salalrajan, F., Seetharaman, K. and Mohanbabu, R. 2004. Evaluation of biocontrol agents against dry root rot of blackgram. *J. Mycol. Pl. Pathol*., 34(2): 341-343.

Sajeena, A., Salalrajan, F., Seetharaman, K. and Mohanbabu, R. 2004. Evaluation of biocontrol agents against dry root rot of blackgram. *J. Mycol. Pl. Pathol*., 34(2): 341-343.

Sandheep, A.R., Asok A.K. and Jisha, M.S. 2013. Combined Inoculation of *Pseudomonas fluorescens* and *Trichoderma harzianum* for Enhancing Plant Growth of Vanilla (*Vanilla planifolia*). *Pakistan Journal of Biological Sciences,* 16: 580-584.

Sethuraman, K., Revathy, N. and Manivannan, M. 2003. Efficacy of bio control organisms on root rot of black gram caused by *Macrophomina phasolina* (Tassi.) Goid. *Legume Res.,* 26(3): 218-220.

Shah, A.R., T.M. Khan, H.A. Sadaqat and A.A. Chatha. 2011. Alterations in leaf pigments in cotton (*Gossypium hirsutum*) genotypes subjected to drought stress conditions. *Int. J. Agric. Biol.* 13: 902-908.

Singh, R.S. 1987. Plant Pathogens (The Fungi). IBH & Oxford Pub. Co. New Delhi.

Sivan, A. and Chet, I. 1989. Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum, J. Gen. Microbiol.,* 135: 675-682.

Subramanian, N., Manoharan, V. and Mariappan, G. 2003. Biocontrol of root rot disease in groundnut. Natl. Sem. on Integrated Plant Disease Management for Sustainable Agriculture, Mar. 20-21, Annamalai University, India, p. 23.

USDA, 2010, www.cnpp.usda.gov/dietary-guidelines-2010

Van Peer, R., Van Kuik, A.J., Rattink, H. and Schippers, B. 1990. Protection of carnation against *Fusarium* by *Pseudomonas* sp. strain WCS417r and Fe-EDDHA. *Netherlands J. Plant Pathol.,* 96: 119-132.

Vesper, S.J. 1987. Production of pili (Fimbriae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. *Appl. Environ. Microbiol.*, 53: 1397-1405.

Zaidi, N.W., Pramila, N. and Singh, U.S. 2004. Biological control of plant pathogens: Status in India. *In:* Singh, S.P. and Singh, S.B. (Eds.), Eco-Agriculture with Bio augmentation: An emerging concept, DASP, Lucknow, pp. 21-52.

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