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Development of *Trichoderma* based bio-formulations for the management of chilli wilt

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Abstract
Locally available carriers were used to develop *Trichoderma* based bio-formulations for the management of chilli wilt. The *Trichoderma* isolate (K2) was isolated locally and were identified as *Trichoderma harzianum* on the basis of cultural and morphological characteristics. Combinations of different carriers with different proportion were found effective in maintaining colony forming unit (cfu) load of *Trichoderma harzianum* for the period of 120 days. Effectiveness of bio-formulations against *Fusarium solani* causing chilli wilt was conducted at two locations which are considered hot spots of *Fusarium solani* causing diverse losses to chilli crop. The maximum inoculum load of 6.22 x 10^8 cfu/g was observed in cow dung-charcoal-molasses (3:2:1v/v) which was followed by cow dung-ash-molasses (3:2:1v/v) with an inoculum load of 6.18 x 10^8 cfu/gram formulation after 30 days of storage. After 120 days (4 months) of observation cfu load of 6.2 x 10^6 was found in cow dung-charcoal-dalweed-soil (3:2:1:1v/v) followed by cow dung-ash-dalweed-soil (3:2:1:1v/v) with an inoculum load of 5.62 x 10^6 cfu/g of formulation. In the field evaluation, seed treatment of *Trichoderma harzianum* (K2) was found effective in managing chilli wilt (37%) followed by seedling dip (29%). Lowest disease control (12%) was found with the seed treatment and seeding dip of carbendazim and captan. *Trichoderma harzianum* was found best in managing wilt diseases of chilli as compared to carbendazim and captan.

Keywords: Biological control, *Fusarium solani*, *Trichoderma*, *Cicer arietinum*, Shelf life

Introduction
Chilli (*Capsicum annuum* L.) known for its pungency is one of the most important vegetable crop grown in all over. Besides pungency the green fruit is also known to contain vitamin A, C and P (rutin) in appreciable proportion (Muthukrishnan *et al*., 2002) [15]. India is the second largest exporter of chilli producing 1.446 million tonnes of ripe dry chillies with an area of 0.869 million ha under its cultivation (Anonymous, 2013; Muthukumar *et al*., 2010) [1, 16]. Amongst the major constraints in chilli production in the state, the losses caused by various soil borne fungal pathogens are devastating affecting the crop right from seed sowing to maturity (Najar, 2001; Sahi and Khalid, 2007; Tayyaba Sultana *et al*., 2014) [17, 23, 28]. The several *Fusarium* species viz., *F. oxysporum* and *F. solani* have been reported as major pathogens causing wilt disease in solanaceous crops (Dwivedi and Enespa, 2013; Yelmame *et al*., 2010) [6, 30]. When the disease occurs in severe form, farmers mostly apply chemical fungicides but due to the high costs and adverse effects on the environment, chemicals are being disregarded.

The biocontrol activity of *Trichoderma* is important not only to agriculture and its crops but also the environment as it does not accumulate in food chain and thus don't harm to the plants, animals and humans (Monte and Llobell, 2003; Perveen and Bokhari, 2012; Reena *et al.*, 2013) [14, 18, 20]. *Trichoderma* as a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention (Choudhary, *et al*., 2013; Santosh reddy *et al*. 2014; Rini and Sulochana, 2007; Tewari and Mukhopadhyay, 2001) [5, 22, 21, 29]. Different organic media like neem cake, coir pith, farmyard manure, and decomposed coffee pulp also have been suggested for its multiplication (Saju *et al*., 2002) [24]. Various substances like pyrax (Beagle-Ristaino and Papavizas 1985) [2], talc (Jeyarajan *et al*., 1994) [10] and alginate pellets (Fravel *et al*., 1985) [7] have been used to formulate the biocontrol agent by different workers. Keeping in view the importance of *Trichoderma* species, the present study was carried to test local isolate of *Trichoderma* (K2) on different locally available carriers for the management of chilli wilt.

Material and methods

Isolation and characterization of bio-control agents

Bio-control agents were isolated from rhizospheric soil of chilli at Shalimar, SKUAST-K. About 250-300 g of soil around roots were collected in a sterile polythene bag and carried to laboratory in a cold container. Isolation of *Trichoderma harzianum* was carried out by dilution plate method (Johnson, 1957), using TSM (*Trichoderma* selective media) as selective media (Elad *et al*., 1981). Samples were plated on TSM and incubated at 25 ± 2^0 C for 3-4 days; the fungal colonies developed in the plate were sub-cultured by single cell isolation on TSM plates. Isolated *Trichoderma* sp. was maintained on TSM slants aseptically at 4^0C. Distinct cultural and morphological characteristics were observed for identification. These include colony growth rate, colony colour, reverse colour, colony edge, mycelial form, conidiophore branching, conidial colour and presence or absence of chlamydospores (Shahid *et al*., 2013) [25].

Fine tuning of bio-formulations

Some commonly available materials i.e., cowdung, ash, charcoal, dal-weed and molasses were mixed in different combinations and evaluated as carriers for isolate K2 (Table - 1). In order to evaluate these carriers on uniform initial moisture level, the air dried mixtures were filled in 250 mL conical flasks to a uniform volume of approximately 100 mL and sterilized in an autoclave before inoculating with 30 mL of uniform spore suspension (2 x 10^8 cfu/mL). The formulations were thoroughly shaken and then kept at ambient temperature (18 - 28^o C) for four months. Observations with respect to viable load colony forming unit (cfu/mL) were recorded during the entire period at an interval of 30 days and every time 1g of formulation was suspended in 100 mL of sterilized water and 1mL aliquot of the homogenate dilutions were spread on solidified PDA surface.

Field evaluation of bio-formulations

The *Trichoderma harzianum* (isolate K2) was evaluated in two chilli fields each at Pattan and Magam of Kashmir. The fields at Pattan and Magam were identified as hot spots of *Fusarium solani*, as the pathogen had devastated the chilli crop in these fields under natural epiphytotic conditions previously. The chilli seedlings were raised in the respective areas as per the following methodology.

T1 = Seed dip in K2 suspension (10^8 cfu /mL), T2 = Seedling dip in K2 suspension (10^8 cfu /mL), T3 = Seed dip in carbendazim suspension (0.1%), T4 = Seedling dip in carbendazim suspension (0.1%), T5 = Seed dip in captan suspension (0.2%), T6 = Seedling dip in captan suspension (0.2%), and T7 = Control (No seed or seedling dip).

Results

Isolation of bio-control agent

The *Trichoderma* sp. (K2) was isolated from rhizospheric soil of chilli at Shalimar, SKUAST-K. After studying the characteristics viz., colony growth rate, colony colour, reverse colour, colony edge, mycelial form, conidiophore branching, conidial colour and presence or absence of chlamydospores *Trichoderma* isolate (K2) was found to resemble *Trichoderma harzianum* (Table 2). The isolate K2 were studied for its multiplication on different locally available substrates with different proportions. Isolate were evaluated at two areas of Kashmir (Magam and Pattan) for the management of *Fusarium solani* responsible for chilli crop loss.

Fine tuning of the bio-formulations

The different combinations of locally available carriers were mixed in different proportions to increase the colony forming unit (cfu) of the bio-formulations. After a storage period of two months at ambient temperature (temperature 10-20 ^{o}C) the formulations showed considerable inoculum load which ranged between 1.6 x 10^6 - 8.8 x 10^7 colony forming units (cfu)/g formulation. The maximum inoculum load (6.22 x 10^8 cfu/g) was observed in cowdung-charcoal-molasses (3:2:1v/v) which was followed by cowdung-ash-molasses (3:2:1v/v) with an inoculum load of 6.18 x 10^8 cfu/g formulation after one month of storage. After four months cfu count was recorded in all the combinations with maximum load (6.2 x 10^6) in cowdung-charcoal-dalweed-soil (3:2:1:1v/v) followed by cowdung-ash-dalweed-soil (3:2:1:1v/v) with a inoculum load of 5.62 x 10^6 cfu/g of formulation (Table 3). Other formulations were found at par with respect to cfu/g of formulation. The charcoal based formulations supported better as compared to ash based formulations. Moreover, the formulations containing soil were better than those containing sand.

Field evaluation of bio-formulations

To evaluate the bio-formulation agent against *Fusarium* wilt of chilli in Pattan and Magam areas of Kashmir different treatments of seed and seedling were carried. The seed dip treatment of *Trichoderma* sp K2 (T1) was found effective in managing chilli wilt (37.36%) followed by seedling dip (T2) of *Trichoderma* species K2 (29.25%). Wilt disease management was found highest at Magam as compared to Pattan. Lowest disease control (12.06%) was carried with seed dip treatment in carbendazim (T3) followed by seed dip treatment in captan (T5) controlled 12.52% of *Fusarium* chilli wilt (Table 4).

Discussion

The present study was planned with the objective to isolate *Trichoderma* species for mass multiplication on different locally available substrates for the management of chilli wilt. Various substrates are being used for the mass multiplication of *Trichoderma* species (Charabarty *et al*., 2014; Kumar *et al*., 2014; Rajput *et al*., 2014; Krishna and Kumar, 2013) [4, 13, 19, 12]. The bio-control agent *Trichoderma* K2 was isolated from rhizospheric soil of chilli at Shalimar, SKUAST-Kashmir. The rhizosphere is known to be a hot spot of microbial activities (Chandrashekar, *et al*., 2014) [4].

Formulation and shelf life are of prime importance for commercial use of any biological agent (Islam *et al*., 2006) [9]. There is abundant literature on the use of conventional synthetic media like glucose, cellulose, soluble starch and molasses to produce *Trichoderma* species (Gupta *et al*., 1997) [8]. The bio-control agent was mixed with different proportions of carriers to increase colony forming unit (cfu) load and was observed upto 4 months. It was found that formulation (FI) cowdung-charcoal-molasses (3:2:1v/v) and (F3) cowdung-ash-molasses (3:2:1v/v) supported the load of colony forming unit (cfu) of *Trichoderma* K2 for first 30 days (1 month) with inoculum load of 6.2 x 10^8 cfu/g and 6.18x10^8 cfu/g respectively. However after 120 days (4 months) of observation formulation cowdung-charcoal-dalweed-soil (3:2:1:1v/v) and (F12) cowdung-ash-dalweed-soil (3:2:1:1v/v) was found effective with maximum inoculum load of *Trichoderma* sp K2 as 6.2x10^6 and 5.62x10^6cfu/g of formulation respectively. *Trichoderma* species have been

multiplied on many carriers by various workers (Saju *et al*., 2002; Jeyarajan *et al*., 1994) [24, 10].

The two areas of Kashmir namely Magam and Pattan were selected for the field trials of bio-control agent as these fields are known hot spots of *Fusarium* wilt of chilli crops. *Trichoderma* K2 was found to be potential in managing the *Fusarium* wilt of chilli at Magam and Pattan. But maximum wilt disease management was found at Magam fields as compared to Pattan. Soil borne plant pathogens such as *Pythium*, *Fusarium* sp., *Rhizoctonia solani, Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Alternaria* sp., *Collectotrichum capsici*, *Phytopthora* sp. and *Meloidogyne* sp. are important pathogens of solanaceous vegetables (tomato, chilli, brinjal) and cause a yield loss of 43-54% in Jammu region (Shali, 2000) [26]. In field evaluation of bio-control agent seed dip treatment of *Trichoderma* sp K2 was found effective in managing chilli wilt (37%) in field. However in earlier studies it has been found that field application of *T. harzianum* at different doses gave 54-86% control of *F. oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* (Kaur and Mukhopadhyay, 1992) [11]. Lowest disease control (12%) was carried with seed dip treatment in carbendazim (T3). Seed treatment with *T. harzianum* or *Paecilomyces lilacinus* controlled the tomato wilt (Sharon *et al.*, 2001) [27].

Table 1: Proportion of different carriers for the evaluation of biocontrol agent

Formulation	Combinations	Proportion (volumes)
F1	Cowdung + Charcoal + Molasses	3:2:1
F2	Cowdung + Charcoal + Dal-weed	3:2:1
F3	Cowdung + Ash + Molasses	3:2:1
F4	Cowdung + Ash + Dal-weed	3:2:1
F5	Cowdung + Charcoal + Molasses + Sand	3:2:1:1
F6	Cowdung + Charcoal + Dal-weed + Sand	3:2:1:1
F7	Cowdung + Ash + Molasses + Sand	3:2:1:1
F8	Cowdung + Ash + Dal-weed + Sand	3:2:1:1
F9	Cowdung + Charcoal + Molasses + Soil	3:2:1:1
F10	Cowdung + Charcoal + Dal-weed + Soil	3:2:1:1
F11	Cowdung + Ash + Molasses + Soil	3:2:1:1
F12	Cowdung + Ash + Dal-weed + Soil	3:2:1:1

Table 2: Cultural and morphological characteristics of *Trichoderma* isolates (K2)

Name of the isolate	Colony growth rate (cm/day)	Colony colour	Reverse colour	Colony edge	Mycelial form	Mycelial colour	Conidiophore branching	Conidial colour
K2	8-9 in 3 days	Dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Highly branched regular	Green

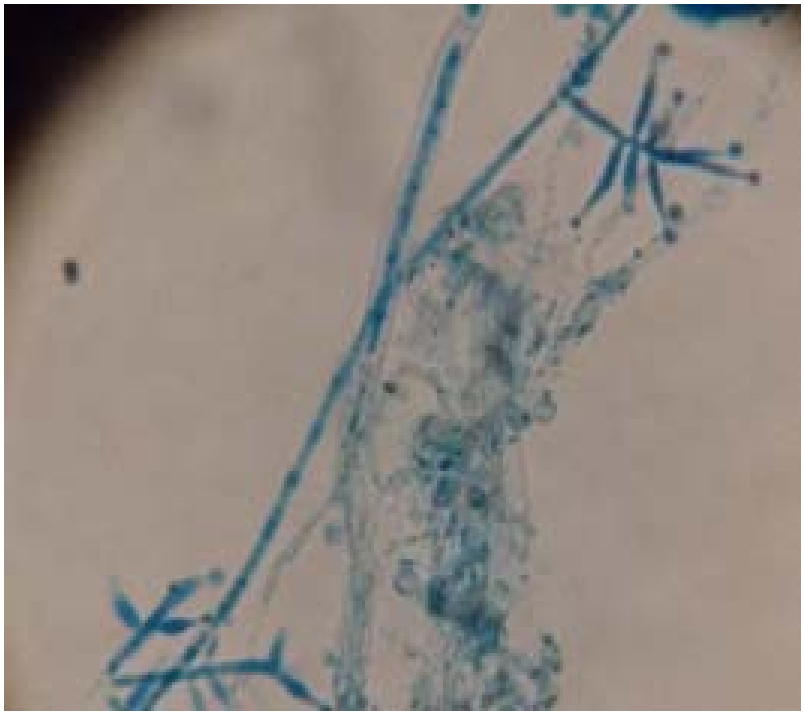
Table 3: Effect of carrier material on shelf life of bio-control agent

Formulation	Viability (cfu/cc)				
	0 day	30 day	60 day	90 day	120 day
F1	1.6×10^6	6.22×10^8	8.80×10^7	8.3×10^7	3.00×10^6
F2	1.6×10^6	6.05×10^8	8.19×10^7	8.0×10^7	4.05×10^6
F3	1.6×10^6	6.18×10^8	7.36×10^7	7.2×10^7	2.12×10^6
F4	1.6×10^6	4.97×10^8	7.93×10^7	$7.9x\ 10^7$	3.30×10^6
F5	1.6×10^6	3.72×10^7	4.89×10^7	3.91×10^7	3.40×10^6
F6	1.6×10^6	3.53×10^7	6.04×10^7	5.42×10^7	4.85×10^6
F7	1.6×10^6	3.66×10^7	4.72×10^7	4.29×10^7	3.31×10^6
F8	1.6×10^6	3.50×10^7	4.32×10^7	3.32×10^7	3.50×10^6
F9	1.6×10^6	3.88×10^7	5.68×10^7	3.18×10^7	5.47×10^6
F10	1.6×10^6	3.79×10^7	5.61×10^7	4.61×10^7	6.20×10^6
F11	1.6×10^6	3.80×10^7	5.35×10^7	3.65×10^7	5.08×10^6
F12	1.6×10^6	3.74×10^7	5.33×10^7	4.13×10^7	5.62×10^6

Table 4: Efficiency of bio-control agent against *Fusarium* wilt of chilli

Treatment	Disease incidence (%)			Av. Disease control over check (%)
	Pattan	Magam	Average	
T1 – Seed dip in K2 (10^8cfu ml^{-1})	30.34	24.49	27.41	37.36
T2 – Seedling dip in K2 (10^8cfu ml^{-1})	32.80	29.12	30.96	29.25
T3 – Seed dip in Carbendazim (0.1%)	37.80	39.12	38.46	12.06
T4 – Seedling dip in Carbendazim (0.1%)	31.25	38.66	34.95	20.13
T5 – Seed dip in Captan (0.2%)	40.26	36.30	38.28	12.52
T6 – Seedling dip in Captan (0.2%)	41.16	34.60	37.28	14.80
T7 – Control (No seed or seedling dip)	48.06	39.50	43.76	-
CD ($P < 0.05$)	2.31	1.96	2.46	

Growth of *Trichoderma harzianum* on PDA



Identification of *Trichoderma harzianum* by conidial formation

References

1. Anonymous. Production and area under chillies and peppers dry in India for the year 2011. Food and Agriculture Organization (FAO), Rome, Italy, 2013.
2. Beagle-Ristaino IE, Papavizas GC. Biological control of *Rhizoctonia* stem canker and black scurf of potato. Phytopathology, 1985; 75:560-564.
3. Chakrabarty R, Acharya GC, Sarna TC. Evaluation of substrates for mass multiplication of bioagent *Trichoderma viride*. African Journal of Agricultural Research. 2014; 9:1938-1940.
4. Chandrashekar MA, Soumya Pai K, Raju NS. Fungal diversity of rhizosphere soils in different agricultural fields. International Journal of Current Microbiology and Applied Sciences. 2014; 3:559-566.
5. Choudhary CS, Jain SC, Ritesh Kumar, Jaipal Singh C. Efficacy of different fungicides, biocides and botanical extract seed treatment for controlling seed-borne

Colletotrichum sp. in chilli (*Capsicum annuum* L.). The Bioscan, 2013; 8(1):123-126.
6. Dwivedi SK, Enespa. *In vitro* efficacy of some fungal antagonists against *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* causing brinjal and tomato wilt. Intern J Biol Pharma Res. 2013; 4:46-52.
7. Fravel DR, Morois JJ, Lumsden RD, Connick WJ. Encapsulation of potential biocontrolagent in an alginate clay matrix. Phytopathology, 1985; 75:774-777.
8. Gupta R, Saxena RK, Goel S. Short communication: Photo induced sporulation in *Trichoderma harzianum*-an experimental approach to primary events. World Journal of Microbiology and Biotechnology. 1997; 13:249-250.
9. Islam MN, Rahman MM, Firoz MJ, Das AK, Amin MW. Influence of temperature and packing materials on shelf-life of mass cultured *Trichoderma* at storage condition. International Journal for Sustainable Crop Production. 2006; 2:01-03.
10. Jeyarajan R, Ramakrishnan G, Dinakaran D, Sridar R. Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot diseases. In: Biotechnology in India. BK Diwivede, eds, Bioved Research Society, Allahabad, 1994, 25-36.
11. Kaur NP, Mukhopadhyay AN. Integrated control of chickpea wilt complex by *Trichoderma* and chemical methods in India. Tropical Pest Management. 1992; 38:372-375.
12. Krishna AH, Kumar MR. *In vitro* screening and evaluation of different substrates and carrier materials for mass multiplication of *Trichoderma* against dry root rot in acid lime incited by *Fusarium solani*. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013; 4:53-60.
13. Kumar S, Roy PD, Lal M, Chand G, Singh V. Mass Multiplication and shelf life of *Trichoderma* species using various agroproducts. An International Quarterly Journal of Life Sciences. 2014; 9:1143-1145.
14. Monte E, Llobell A. *Trichoderma* in organic agriculture. V Congreso Mundial del Aguacate, 2003, 725-733.
15. Muthukrishnan CR, Thangaraj T, Chatterjee R, Maity TK. Capsicum and chilli. In: (eds. T.K. Bose, J. Kabir, T.K. Maity, V.A. Parthasarathy & M.G. Som) Vegetable Crops. Naya Porkash, Kolkata, India. 2002, 204-261.
16. Muthukumar A, Eswarana A, Nakkeeranb S, Sangeethaa G. Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. Crop Protect, 2010; 29:1483-1488.
17. Najar AG. Cause and management of chilli wilt in Kashmir. PhD thesis, S.K. University of Agricultural Science and Technology of Kashmir, Shalimar, Srinagar Jammu and Kashmir, India, 2001.
18. Perveen K, Bokhari NA. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum.* African Journal of Microbiology Research. 2012; 6:3348-3353.
19. Rajput AQ, Khanzada MA, Shahzad S. Effect of different substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma pseudokoningii.* International Journal of Agriculture and Biology. 2014; 16:893-898.
20. Reena A, Anitha M, Aysha OS, Valli S, Nirmala P, Vinothkumar P. Antagonistic activity of *Trichoderma viride* isolate on Soil borne plant pathogenic fungi. International Journal of Bioassays. 2013; 2:294-297.
21. Rini CR, Sulochana KK. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. Journal of Tropical Agriculture. 2007; 45:21-28.
22. Santosh reddy, Machenahalli, Nargund VB, Hegde RV. Management of fruit rot causing seed borne fungal pathogens in chilli. The Bioscan. 2014; 9(1):403-406.
23. Sahi IY, Khalid AN. *In vitro* biological control of *Fusarium oxysporum* causing wilt in *Capsicum annuum*. Mycopath, 2007; 5:85-88.
24. Saju KA, Anandaraj M, Sarma YR. On farm production of *Trichoderma harzianum* using organic matter. Indian Phytopathology, 2002; 55:277-281.
25. Shahid M, Srivastava M, Sharma A, Kumar V, Pandey S, Singh A. Morphological, molecular identification and SSR Marker analysis of a potential strain of *Trichoderma*/Hypocrea for production of a bioformulation. Journal of Plant Pathology and Microbiology. 2013; 4:10.
26. Shali SK. Studies on chilli (*Capsicum annum* L.) wilt in Jammu. M. Sc. Thesis. Division of Plant Pathology, SKUAST, Jammu, 2000.
27. Sharon E, Bar-Eyal M, Chet I, Herrera-Estrella A, Kleifeld O, Spiegel Y. Biological control of the root knot nematode *Meloidogyne javanica* by *Trichoderma harzianum.* Phytopathology, 2001; 9:687-693.
28. Tayyaba Sultana, Farah Naz, Haq MIU, Butt S, Abas MF. Characterization and relative contribution of fungal and bacterial pathogens involved in sudden death syndrome of chillies. Pak. J. Phytopath. 2014; 26:53-61.
29. Tewari AK, Mukhopadhyay AN. Testing of different formulations of *Gliocladium virens* against chickpea wilt complex. Indian Phytopathology, 2001; 54:67-71.
30. Yelmame MG, Mehta BP, Deshmukh AJ, Patil VA. Evaluation of some organic extracts in *in vitro* to control *Fusarium solani* causing chilli wilt. Intern. J Pharma Bio-Sci. 2010; 6:122-126.