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Ritu Mawar, A. S. Tomer & Dheeraj Singh

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RESEARCH ARTICLE

Demonstration of efficacy of bio-control agents in managing soil-borne diseases of various crops in arid region of India

Ritu Mawar[1] · A. S. Tomer[2] · Dheeraj Singh[3]

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Abstract
Effectiveness of bio-formulated products of bio-agents *Trichoderma harzianum, Bacillus firmus* and consortium of both as seed treatment were demonstrated at farmer's field against dry root rot of guar, moth bean, mung bean and sesame caused by *Macrophomina phaseolina* during rainy seasons of 2007–2016. The efficacy of T. *harzianum* was also demonstrated against wilt of cumin caused by *Fusarium oxysporum* f sp. *cumini*. Studies revealed that seed treatment with bio-agents significantly reduced incidence of dry root rot on legumes (4.7%), oilseeds (21.6%) and that of wilt on cumin (22.6%) in all the demonstrations resulting in significant increase in seed yield. Maximum yield promotion (14.9–19.0%) due to seed treatment was recorded in sesame during both the years at villages of Pali district. Similarly, significant reduction in wilt incidence and thereby increased seed yield (14.5–23.1%) was recorded in cumin at both the districts of Rajasthan. Consortium of both the bio-agents also increased seed yield by 22.9% in guar at Jodhpur. These demonstrations have resulted in wider acceptance by rain-fed farmers and more by cumin growers under irrigated conditions.

Keywords *Trichoderma harzianum* · *Bacillus firmus* · *Macrophomina phaseolina* · *Fusarium*

Introduction

In Indian arid region, guar{*Cymopsis tetragonoloba* (L.) Taub.,} moth bean (*Vigna acontifolia* Jacq.), cowpea {*Vigna unguiculata* (L.)Walp}, mung bean (*Vigna radiata* L.) and sesame (*Sesamum indicum* L.) are grown during rainy season. Under warm growing conditions these crops experience long and short duration of moisture stress. In this situation, soil borne plant pathogen, *Macrophomina phaseolina* (Tassi) Goid., causes dry root rot in these crops. Losses to the tune of 40% have been reported in cowpea alone due to dry root rot disease (Lodha et al. 1986). This pathogen survives in the soil primarily as sclerotia, and their population increases in the soil with each year of cultivation of susceptible crops and the inoculum density is directly proportional to the disease intensity in the field. In irrigated pockets of the region, cumin (*Cuminum cyminum* L.) is cultivated during winter season. However, this crop suffers heavily by wilt caused by *Fusarium oxysporum* f. sp. *cumini* (Lodha and Mawar 2014). Several management strategies like moisture conservation practices (Lodha 1996), soil solarization (Lodha 1995), *Brassica* residues (Mawar and Lodha 2002), composts (Bareja et al. 2013) and bio control agents (Lodha et al. 2013) have been advocated for managing soil borne plant pathogens in arid region. In recent years, native strains of bio agents' viz., *Bacillus firmus* and *Trichoderma harzianum* have been isolated from arid soils. *B. firmus* was found specific antagonist to *M. phaseolina* in various laboratory tests (Lodha et al. 2013). Once a bio-control agent has shown reproducible activity in screening trials, methods for inoculum production, formulation and application needs to be considered in relation to the crop, disease and environment of use. Therefore, efforts were made to develop bio-formulated products of these bio-pesticides. After developing bio-formulated products, it was thought worthwhile to test their efficacy at growers' field in order to disseminate this cheap and easy management strategy in the region and to collect feedback information. Bioformulated products of *T. harzianum*, *B.firmus* and consortium of *T. harzianum* + *B.*

✉ Ritu Mawar
ritumawar1976@gmail.com

[1] ICAR-Central Arid Zone Research Institute, Jodhpur 342003, India

[2] KVK, ICAR-CAZRI, Jodhpur, India

[3] KVK, ICAR-CAZRI, Pali, India

firmus were coined as Maru sena 1, 3 and Mishrit sena, respectively.

Materials and methods

All the laboratory studies were conducted at the Plant Pathology Laboratory of the Central Arid Zone Research Institute, Jodhpur, while field demonstrations were carried out at adopted villages of the Institute, KVK Jodhpur and Pali districts of Indian arid region with a previous history of cultivation of legumes, sesame or cumin. The quantum of rainfall and its distribution varies in both the districts, which results in appearance of disease incidence on legumes grown under rain fed conditions at different stages of crop growth reflected in seed yield. Soil samples were collected from 0 to 30 cm soil depth at some of the locations, air dried and processed for determination of pathogenic propagules. The sclerotial population of *M. phaseolina* ranged between 123 and 320 g^{-1} soil while, population of *Fusarium* in cumin infested fields ranged between $8.9–21.3 \times 10^3$ cfu^{-1} soil at 0–30 cm soil depth as determined on their respective selective media (Mayer et al. 1973; Papavizas, 1967).

Laboratory study

Mass production of bio-control agents

T. harzianum A pure culture of *T. harzianum* isolated from native soil of the region was multiplied in several 250 ml flasks containing 150 ml each of autoclaved potato dextrose broth for 7 days. Fungal mats were harvested after 7 days and blended for 30 s in 200 ml of distilled water. This suspension having 5.6×10^9 cfu ml^{-1} of fungal bio-control agent prepared in distilled water was mixed with food substrate like residues of on-farm waste and inert materials. The solid carrier material was grounded-up separately and air dried. The sterile products having bio agent were packed in 50–70 µm thick low density polyethylene bags to protect from loss of moisture. The bags were sealed leaving about 25% air space to give proper aeration to the inoculant. The initial count in carrier-based preparation was made so as to obtain 10^8 cells g^{-1} at the time of distribution to farmers.

B. firmus The bacterium was multiplied in 250 ml flasks containing 150 ml of Czapeck dox broth for 7 days and then blended by a hand grinder. Suspension had 6×10^8 cfu ml^{-1} of bacterial mass, which was then mixed with food substrates and lignite as an inert material for maintaining 35% moisture of MHC for a period of 120 days. The solid carrier material was grounded-up separately and air dried before mixing. The sterile products having bio agents were packed as described for *T. harzianum*.

Consortium of *T. harzianum* + *B. firmus* Both the bio-agents were multiplied separately as described above and were mixed in food substrates, which supports survival and multiplication of both the bio-agents and packed as described in case of *T. harzianum*.

Field demonstrations

T. harzianum (Maru sena 1)

Effectiveness of seed coating with bio formulated product of *T. harzianum* on incidence of dry root rot and seed yield of Guar, moth bean and sesame and on incidence of wilt on cumin were demonstrated at 179 grower's field during 2015–2016. Guar, moth bean Sesame and cumin seeds were coated with bio-formulated product at @4 gm^{-1} kg seeds at adopted villages of the Institute and Krishi Vigyan Kendras (KVKs) of Jodhpur and Pali districts in the presence of growers in order to simultaneously impart training. At each demonstration, 800 m^2 areas each were planted with bio-agent coated and uncoated seeds within 24–48 h of coating. Plant mortality due to dry root rot and incidence of wilt were recorded 55–60 days after growth in 5 randomly selected plots (4 × 4 m) at each site. Seed yield (kg ha^{-1}) was also recorded after harvesting. Incidence of diseases and yield were pooled for each crop.

B. firmus (Maru sena 3)

Effectiveness of seed coating with bio formulated product of *B. firmus* on incidence of dry root rot and seed yield of Guar and moth bean was demonstrated at 61 growers field adopted by the Institute during 2007–2009 in the same manner as described for *T. harzianum* except that the mixer of bio formulated product was dissolved in jaggary suspension before coating the seeds. The area of demonstration was same as described for *T. harzianum*. Plant mortality due to dry root rot and seed yield was also recorded as described for *T. harzianum*.

Consortium of *T. harzianum* + *B. firmus* (Mishrit Maru sena)

Effectiveness of seed coating with bio formulated product of consortium of *T. harzianum* + *B. firmus* on incidence of dry root rot and seed yield of Guar were demonstrated at 10 growers field during 2015–2016 in the same manner as described for *B. firmus*. The area of demonstration and data recording also remained same as described for *T. harzianum*.

Statistical analysis

All the data were analysed statically as per location irrespective of the crop and the year. Incidences of diseases and seed yield were subjected to analysis and the treatment means were compared after performing *t* test and coefficient of variations were determined as per standard procedure (Snedecor and Cochran 1967). Data on per cent disease incidence were not subjected to angular transformation due to variation in disease incidence below 20%. Per cent reduction in disease incidence was calculated by subtracting disease incidence in treated and control plots.

Results and discussion

T. harzianum (Maru sena 1)

Seed treatment with bio-agent reduced incidence of dry root rot and increased the seed yield of guar, moth bean and mung bean at all the villages where demonstrations were conducted by KVK Jodhpur during 2015 and 2016. In 2015, incidence of dry root rot ranged from 8.1 to 11.6% with minimum being in treated and maximum in untreated plots in 20 demonstrations (Table 1). On the basis of pooled average of all the demonstrations conducted on guar resulted in 13.3% yield promotion. In 2016 also, there was significant reduction ($t=0.05$) in dry root rot incidence in guar, moth bean and mung bean with minimum being in moth bean. Accordingly, yield promotion was more discernible in guar compared to moth bean (Table 1). Studies on water relation parameters have shown that cowpea followed by guar are more vulnerable to *M. phaseolina* compared to moth bean (Burman and Lodha 2000). At the adopted villages of KVK Pali, significant reduction in diseases incidence and increase in seed yield was recorded at all the 41 demonstrations conducted on guar, mung bean, moth bean, cowpea and sesame crops in 2015 and 2016. The per cent increase in seed yield just by seed treatment was 13.3–16.4% (guar), 11.1–20% (mung bean), 14.9–15.2% (moth bean), 25.3% (cowpea) and 14.9-19.0% (sesame) in different demonstrations. Significant increase in seed yield at three sesame locations of Pali in 2016 could be attributed to severe moisture stress faced by crop at flowering stage onwards leading to increased incidence of dry root rot in control plots. Seed treatment with bio-agent resulted in considerable reduction in incidence of wilt on cumin crop at 25 demonstrations conducted by KVK Pali and Jodhpur during winter season of 2015–2016 resulting in 14.5–23.1% yield promotion (Table 1). *T. harzianum* is a proven bio-agent against many fungal plant pathogens particularly soil borne plant pathogens like *Macrophomina, Fusarium, Pythium*, etc. In earlier studies carried out at the Institute by the same native strain considerable reduction in dry root rot and wilt incidence was recorded on legumes, oil seeds and cumin (Isreal et al. 2005; Lodha and Mawar 2010).

Table 1 Effectiveness of bio-formulated product of Trichoderma harzianum (*Maru sena* 1) on dry root rot incidence and wilt of cumin with seed yield

Crop	No. of demonstrations[a]	Disease incidence (%)		Yield (kg/ha)		Increase in yield (%)
		Treated	Control	Treated	Control	
Jodhpur						
2015	20	8.1	11.6*	340	300*	13.3
Guar	22	13.5	20.6*	687	600*	14.5
Cumin						
2016	62	7.1	11.5*	400	345*	13.0
Guar	15	7.4	12.5*	402	360*	11.6
Moth	15	8.5	15.7*	340	300*	13.3
Mung bean		28.9	26.6	31.6	30.5	
CV (%)						
Pali						
2015	4	6.1	8.0*	510	450*	13.3
Guar	4	7.2	9.5*	640	557*	14.9
Moth	5	6.4	10.5*	870	783*	11.1
Mung bean	5	8.4	11.4*	640	555*	14.9
Sesame	3	16.1	33.6*	850	690*	23.1
Cumin						
2016	5	4.7	17.5*	1130	970*	16.4
Guar	4	4.9	16.4*	680	590*	15.2
Moth	5	5.5	14.7*	1140	950*	20.0
Mung Cowpea	4	7.3	11.8*	1580	1260*	25.3
Sesame	5	9.7	20.5*	750	630*	19.0
CV (%)		35.4	23.1	32.3	29.6	

*Significant (p=0.05) on the basis of *t* values

[a]Pooled average of all the demonstrations in different villages of Jodhpur and Pali districts for all crops

B. firmus (Maru sena 3)

Least plant mortality 6.81% due to dry root rot in guar was recorded on the basis of pooled average of 22 locations where seeds were coated with bio agent compared to 10.8% in plots sown with uncoated seeds (Table 2). The effect of seed coating was more conspicuous on local cultivar than improved short duration genotypes. Increase in seed yield in the plots planted with *B. firmus* coated seeds ranged from 14.8 to 17.5% in guar and 14.5% in moth bean, respectively. In general, better germination and early flowering has been witnessed by growers in treated plots indicating that increase in seed yield was not only due to reduced disease incidence but was also due to cumulative effect of better germination, plant growth and nodulation. Greater coefficient of variance (42.4–47.3%) observed with this bioformulated product could be attributed to quantum and distribution of rainfall, which results into occurrence of disease at different stage of the crops. A number of *Bacillus* species like *B. subtilis* and *B. cepacia* are also known to control *Pythium, Fusarium* species and *Rhizactonia solani* (Sharma and Sharma 2007). This bacterium is omnipresent in arid soils. *B. firmus* is an established phosphate solubilizing bacterium (Banik and Dey1982) and in our earlier studies, its growth promoting characteristics has also been established (Lodha et al. 2013). Nitrogen fixing ability of other spp. of *Bacillus* native to arid soils of India has been observed by Bajoria et al. (2008).

Consortium of *T. harzianum* + *B.firmus* (Mishrit Maru sena)

Bio-formulated product of consortium demonstrated at 10 farmers field by KVK Jodhpur resulted in significant reduction ($t = 0.05$) in disease incidence (7.6%) compared to control (12.2%) resulting in 22.9% increase in seed yield of guar on the basis of pooled average (Table 3). Compatibility of both the bio-agents was tested in earlier studies (Lodha et al. 2013). This bio-formulated product also improved root colonization of *B. firmus* providing long duration of protection to host by pathogen (Mawar and Lodha 2012). Its application as seed coating along with incorporation of small dose of compost prepared from radish residues further improved root colonization. *T. harzianum* employs various mechanisms like mycoparasitism, antibiosis and competition to colonize hyphae and sclerotia of pathogen, ultimately causing host death, while *B. firmus* act through antibiosis thus both providing better protection to host compared to their use alone. Singh et al. (2013) also reported that seed and seedling treatment with consortium of *Pseudomonas florescence* and *T. harzianum* resulted in plant growth promotion, yield and simultaneously reduce the disease severity due to *Sclerotium rolfsii* in tomato in contrast to application of individual bio agent. Application of *Trichoderma* formulations with strain mixtures also performed better than individual strains in management of diseases (Kumar et al. 2017). Patel et al. (2016) also observed that a mixture of fungal and bacterial antagonist were found significantly superior in control of *Erysiphae pisi*, as observed in the present study. *Bacillus*

Table 2 Effectiveness of bio-formulated product of *Bacillus firmus* (*Maru sena* 3) on dry root rot incidence and seed yield of arid legumes

Crop	No. of demonstrations[a]	Disease incidence (%)		Yield (kg/ha)		Increase in yield (%)
		Treated	Control	Treated	Control	
Jodhpur 2007	22	6.8	10.8*	331	282*	17.5
Guar						
Jodhpur 2008	33	10.5	16.5*	352	306*	14.8
Guar	6	20.4	29.2*	476	416*	14.5
Moth bean		47.3	42.4	17.2	18.0	
CV (%)						

*Significant (p = 0.05) on the basis of *t* values

[a]Pooled average of all the demonstrations in different villages of Jodhpur district for all crops

Table 3 Effectiveness of consortium of bio agents (*Mishrit maru sena*) on dry root rot incidence and seed yield of guar in 2015

Crop	No. of demonstrations[a]	Disease incidence (%)		Yield (kg/ha)		Increase in yield (%)
		Treated	Control	Treated	Control	
Guar	10	7.6*	12.2*	750*	610*	22.9
CV (%)		12.3	8.3	2.3	2.2	

*Significant (p = 0.05) on the basis of *t* values

[a]Pooled average of all the demonstrations in different villages of Jodhpur district for all crops

spp. has characteristics particularly suited for studies on biological control: omnipresence in soils, high thermal tolerance, rapid growth in broth culture and ready formation of resistant spores. In general, effect of seed coating was more conspicuous on sesame and cowpea confirming our field observations that under similar growing conditions these crops are more vulnerable to dry root rot compared to short duration legumes. Earlier five formulations of *Aspergillus niger* coined as Kali sena were developed for the control of several soil borne plant pathogens and large scale field testing was done (Sen 2000).

Our endeavour to demonstrate use of these products of bio agents resulted into great success in order to disseminate a non- chemical, cost effective and easy in application strategy to manage soil borne plant pathogens of arid crops. Resource deficient growers are not able to accept technologies where lot of water and expensive external inputs are used, but this is a Low Input Sustainable Agriculture (LISA) technology. Use of bio agents does not require any expensive resources; more so, these products are readily available and can be used by farmers just after an initial on- farm training. These products are made available for distribution to the farmers through ATIC of the institute at reasonable cost. In the last 8 years, more than 4000 hectares of land was sown with bio-agents coated seeds in the region. Cumin is exclusively cultivated in Rajasthan and Gujarat states of India. India exports this commodity to more than 70 countries. Often farmers have to abandon their fields from cumin cultivation due to severe occurrence of wilt. Use of bio agent for seed treatment will certainly boost up cumin cultivation in both the states. Our efforts culminated in identifying suitable food substrates, which were combined with carrier to retain moisture in the bio-formulated product so that bio agents can survive for a period of 120 days. The process of developing bio-formulated product of these bio-pesticides has been published by Indian patent site in order to put these products for commercialization for wider adaptability among growers. Demonstrations of these products at growers' field as an essential component of any technology have given encouraging results by way of reducing disease incidence and in increasing yield.

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References

Bajoria S, Varshney AK, Pareek RP, Mohan MK, Ghosh P (2008) Screening and characterization of antifungal guar (*Cyanmoppsis tetragonoloba*) rhizobacteria. Biocontrol Sci Technol 18:139–146

Banik S, Dey BK (1982) Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate solubilizing micro-organisms. Plant Soil 69:353–364

Bareja M, Mawar R, Mathur M, Lodha S (2013) On-farm waste-based composts suppressive to *Macrophomina phaseolina* inciting dry root rot of guar in an arid environment. Aust J Plant Pathol 42:9–16

Burman U, Lodha S (2000) *Macrophomina* infection and expression in relation to plant water status of arid legumes. J Natl Bot Soc 54:13–18

Israel S, Mawar R, Lodha S (2005) Soil solarization, amendments and bio-control agents for the control of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* in aridisols. Ann Appl Biol 146:481–491

Kumar G, Maharshi A, Patel J, Mukherjee A, Singh HB, Sarma BK (2017) *Trichoderma*: a potential fungal antagonist to control plant diseases. Ann Tech Issue 21:206–2018 **(ISSN 0971-975X)**

Lodha S (1995) Soil solarization, summer irrigation and amendments for the control of *Fusarium oxysporum* f. sp. *cumini* and *Macrophomina phaseolina* in arid soils. Crop Prot 14:215–219

Lodha S (1996) Influence of moisture conservation techniques on *Macrophomina phaseolina* population, dry root rot and yield of cluster bean. Indian Phytopath 49:342–349

Lodha S, Mawar R (2010) Efficacy of native bio-control agents on soil microflora, dry root rot incidence and seed yield of rainfed arid crops. Indian Phytopath 63:195–198

Lodha S, Mawar R (2014) Cumin wilt management—a review. J Spices Aromatic Crops 23:145–155

Lodha S, Gupta GK, Singh S (1986) Crop disease situation and some new records in Indian arid zone. Ann Arid Zone 25:311–320

Lodha S, Mawar R, Chakarbarty PK, Singh Bhagwan (2013) Managing *Macrophomina phaseolina* causing dry root rot of legumes by a native strain of *Bacillus firmus*. Indian Phytopath 66:356–360

Mawar R, Lodha S (2002) *Brassica* amendments and summer irrigation for the control of *Macrophomina phaseolina* and *Fusarium* in hot arid region. Phytopath Medditt 41:45–54

Mawar R, Lodha S (2012) Synergistic effect of bacterial and fungal antagonist for improving control of Macrophomina phaseolina causing dry root rot on arid crops. In: 2nd international symposium on biopesticide and ecotoxicological network held at Kasetsart University, Bangkok Thailand from Sep 24–26

Mayer WA, Sinclair JB, Khare MN (1973) Biology of *Macrophomina phaseolina* in soil studied with selective media. Phytopathology 63:613–620

Papavizas GC (1967) Metabolites of *Fusarium oxysporum* f. sp. *cumini* in relation to cumin wilt. Indian Phytopath 18:335–339

Patel JS, Sarma BK, Singh HB, Upadhyay RS, Kharwar RN, Ahmed M (2016) *Pseudomonas fluorescens* and *Trichoderma asperellum* enhance expression of Gá subunits of the pea heterotrimeric G protein during *Erysiphe pisi* infection. Front Plant Sci 6:1206

Sen B (2000) Biological control: a success story. Indian Phytopath 53:243–249

Sharma S, Sharma AK (2007) Biocontrol- An eco-friendly disease management approach. In: Trivedi PC (ed) Bio control of plant diseases. Publishers, Aavishkar, pp 1–19

Singh SP, Singh HB, Singh DK (2013) *Trichoderma harzianum* and *Pseudomonas* sp mediated management of *Sclerotium rolfsii* rot in tomato (*lycopersicon esculentum* mill). Bioscan 8:801–804

Snedecor GE, Cochran WJ (1967) Statistical methods. Oxford & IBH publishing Co., Calcuttaa, pp 258–298