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Seed Bio-priming Mediated Control of Cercospora Leaf Spot and Bacterial Wilt Disease Resistance in Chilli (*Capsicum annuum* L.) under New Alluvial Zone

Desetty JMSNK Sri Veda[1], Pravallika Sree Rayanoothala* and Prabir Chakraborti[2]

[1,2]Department of Seed Science and Technology, BCKV, Mohanpur, Nadia, W.B., India
****Department of Plant Pathology, BCKV, Mohanpur, Nadia, W.B., India***

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ABSTRACT

The potential bio-control agents *Trichoderma harzianum*, and *Pseudomonas fluorescens* in alone or combination were used as seed bio-priming for reviewing their efficacy in reduction of the devastating diseases, the *Cercospora* leaf spot (*Cercospora capsici*) and Bacterial wilt (*Ralstonia solanacearum*) of chilli (*Capsicum annuum L.*). Considering two important seasons, the Rabi season was comparatively vital as its disease severity was more. In chilli, the disease index was significantly reduced in combining the *Trichoderma harzianum* + *Pseudomonas fluorescens* treated seeds, followed by the *T. harzianum* treatment compared to the control (hydro-primed) against cercospora leaf spot of chilli. In case of Bacterial wilt, it was found that T3 followed by *Pseudomonas fluorescens* treated seeds. Chilli seeds were primed with *Trichoderma harzianum* + *Pseudomonas fluorescens* revealed maximum disease protection that was displaying favour in pre-kharif and early stage (35DAS) in comparison to rabi and 60DAS of chilli production. The ability of genotype indicated variation where the distinct variation was noticeable in V2 (G-4) mentioning its high tolerance at 35DAS. The application of combined bio-priming may be suitable to control both *Cercospora* leaf spot and Bacterial wilt of chilli.

***Key words**: Biopriming, Trichoderma, Pseudomonas, Chilli, Cercospora, Bacterial wilt*

Introduction

Chilli (*Capsicum* sp.) is a flowering herbaceous plant of the Solanaceae family. It is believed to have originated in South and Central America, where it has been grown for thousands of years. For its tasty, spicy fruits, the crop is widely cultivated throughout tropical Asia and equatorial America. Although it is a self-pollinated crop, bees, ants, and thrips may cause significant cross-pollination (up to 10%) owing to wandering. Chilli output in 2013 was 3.91 million hectares, with an average dry chilli fruit yield of 1.78 t/ha (FAO, 2013). In 2017-18, India's chilli crop covered 309 thousand hectares, with a yield of 3592 million tonnes (Department of Agriculture Cooperation and Farmer Welfare). West Bengal is the largest state in India in terms of size, output, and productivity, with 70,000 hectares, 110 metric tonnes, and 1.57 metric tonnes per hectare (Ministry of Agriculture and Farmers welfare, GOI, 2016-17).

Agriculture's primary goal is to produce seeds and fruits. As a result, it's vital to maintain the seed/fruit quality by applying natural therapies that provide a variety of advantages. Seed priming, which includes hydro priming, Osmo priming, solid matrix priming, hormo-priming, chemo-priming, nutripriming, and biopriming, is one of the many ways that may be employed to promote seed germination, seedling vigour, and abiotic stress resistance. In addition to these, the biopriming approach offers the benefit of biotic stress management. Biopriming is emphasised as a viable technology alternative to achieve the three-part goal viz. food-nutritional security, environmental stewardship/quality, and agricultural profitability in various agroecosystems.

Bio-priming is the recent practice for controlling major seed and soil borne pathogens and to promote more uniform seed germination/ plant growth associated with fungi and bacteria coatings (Entesari *et al.,* 2013). Seed priming with bio-inoculants helps in disease suppression by utilizing different mechanisms such as siderophore production, antimicrobial secondary metabolite and secretion of lytic enzymes (Keswani *et al.*, 2014). Furthermore, the bio-priming approach has the added benefit of biotic stress management. It also concerns regarding the input's energy-intensive production, as well as the risk to human health from indiscriminate use, pave the way for bio-inoculants to be exploited as a viable link for improved utilisation and use efficiency. Beneficial bacteria and fungi contribute to plant growth and development through root colonisation, the production of siderophores and hormones, and nutrient absorption processes (Gowtham *et al*., 2018). Siderophores are small, high-affinity iron chelating compounds secreted by bacteria and fungi and considered as one of the strongest soluble Fe 3+ -binding agents mediated by several strains of *Pseudomonas* that control biologically soil borne pathogens when applied as seed inoculants to agricultural crops (Burr and Caesar, 1984). When compared to chemical treatment on the plant, it showed that multiple modes of action, such as antibiosis, synthesis of essential secondary metabolites, cell wall degrading enzymes, callose and lignin deposition in the cell wall, resulted in a reduction of disease severity and induction of systemic resistance in the plant via sending signals from root to shoot system under long-term protection against invading pathogens (Labuschagne *et al*., 2010). The *Pseudomonas* siderophores reported to suppress disease and enhance plant growth by the production of fluorescent siderophores that chelate molecular iron in rhizosphere (Singh *et al.,* 2011; 2014; Jain *et al*., 2012). *Trichoderma* spp. are soil-dwelling fungi that can inhibit the growth and development of soil-borne diseases by a number of processes, including mycoparasitism. The production of cell wall degrading enzymes and antibiotics, rhizospheric competition for space and nutrients, and the induction of systemic resistance in chilli, pea, chickpea, tomato, and other plants (Saxena *et al*., 2020) were also observed.

Chilli production is hampered by the emergence of a variety of illnesses caused by fungus, bacteria, viruses, nematodes, and abiotic stressors. Cercospora leaf spot caused by *Cercospora capsici* is the most important production restrictions in the chilli industry in India, resulting in an unavoidable decline in both quantity and quality of fruit output. Both of these illnesses are common in tropical and sub-tropical climates. Cercospora leaf spot is more common when the temperature is less than 28°C, the relative humidity is less than 92 percent, and the pH is between 5 and 6. Below 90% RH, the illness does not develop. The fungus lives on plant waste, with main infection originating from spores released into the air (Islam *et al*., 2016).

The symptoms of bacterial wilt include withering of top leaves that progresses to full wilting of the plant within a few days, as well as evidence of stunting and adventitious root formation in the main stem. *R. solanacearum* has a polar flagellar tuft and is aerobic, Gram-negative, rod-shaped, motile, and non-fluorescent. White fluidal colonies with pink centre are virulent, whereas red colonies are avirulent. Bacterial wilt is more frequent in northern areas, although it is more severe in tropical, subtropical, and temperate areas with hot, humid summers. *R. solanacearum* is a seed-borne bacterium that may survive for years in soil and agricultural residues (Denny, 2006).

Materials and Methods

An *in vivo* experiment was conducted at Teaching Farm, Mandouri, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India, between 2020 and 2021. *Trichoderma harzianum* and *Pseudomonas fluorescens* strains were employed separately and in combination with a control (Hydropriming), and each was duplicated three times. For seed bio-priming, talc-based formulations of Trichoderma spp. and Pseudomonas spp. containing 108cfu/gm were utilised. Masinga morok (V1) and G4 (V2) were significant genotypes of Chilli (*Capsicum annuum* L) that were sown in the field throughout two seasons, pre-kharif and rabi, for this study. After surface cleaning with 0.1 percent HgCl2, the healthy seeds were used. he seeds were primed through soaking of formulated product of different bio-inoculants @ 10g/kg seed for overnight, maintaining high humidity and shady atmosphere afterwards. Then seeds were sown in nursery beds to develop the healthy seedlings. The considerable treatments denoted as T1: *Trichoderma harzianum*; T2: *Pseudomonas fluorescens*; T3: *T. harzianum+ P. fluorescens*; T4: Hydro-priming (Control).

By choosing 5 plants/plot for each replication, the Percent Disease Index under cercospora leaf spot and bacterial wilt disease of chilli was recorded. The three observations were made on trifoliate leaves from the plant's base, middle, and top portions at 35 DAT and 60 DAT during the Pre-kharif and Rabi seasons. The standard graded scale of 0-5 is used for observations (Mathur *et al*., 1972) was used which was utilised for calculating the per cent disease Indexby the following formula-

$$\text{PDI} = \frac{\Sigma \text{ of ratings of infected leaves observed}}{\text{No. of leaves observed x maximum disease score}} \times 100$$

Results and Discussion

The Percent Disease Index (PDI) on Cercospora leaf spot and Bacterial wilt of chilli was detected in this experiment using two genotypes in two seasons at 35 and 60 DAT stages. Variation was seen among genotypes, seasons, and plant development stages. The data on PDI on Cercospora leaf spot was presented in Tables 1 and 2, and PDI was presented in Tables 3 and 4, where the recorded value indicated a significant superior demarcation in application of different treatments as priming over control under both 35 DAT and 60 DAT stages of the chilli plant. Considering the different treatments, the combining effect of *T. harzianum* and *P. fluorescens* displayed lowest PDI indicating its super most effect to control the disease in both pre- kharif to rabi seasons though pre-kharif was dominant to control it.

Tables 1 and 2 show that there was a higher incidence of disease in 60 DAT when both genotypes and seasons were considered. The incidence of disease responses followed a similar pattern, with *T. harzianum* and *P. fluorescens* having similar effects. When comparing the pre-kharif and rabi results, the two genotypes exhibited a difference in their disease incidence pattern, with the Masinga morok (V1) genotype exposing a higher PDI, indicating its greater sensitivity to disease, which was seen in both pre-kharif and rabi seasons.

The higher bacterial wilt incidence of disease was monitored in 60 DAT, considering both genotypes and seasons, as shown in Tables 3 and 4. The incidence of disease responses followed the same pattern, with *T. harzianum + P. fluorescens* having a more or less identical impact, which was followed by *P.*

Table 1. Effect of bio-priming in incidence of Cercospora leaf spot in chilli at 35 DAT considering two seasons

Treatments	Masingamorok		G-4	
	Pre-kharif	Rabi	Pre-kharif	Rabi
Trichoderma harzianum	41.54	50.20	18.14	28.65
Pseudomonas fluorescens	42.91	51.57	19.51	30.02
T. harzianum+ P. fluorescens	41.88	50.54	18.48	28.99
Hydropriming (Control)	60.58	69.24	37.18	46.55
C.D. at 5%	6.16	6.26	7.16	5.58
CV%	7.01	5.91	14.03	8.84

The values mentioned above were the mean of Percent disease index three replications

Table 2. Effect of bio-priming in incidence of Cercospora leaf spot in chilli at 60 DAT considering two seasons

Treatments	Masingamorok		G-4	
	Pre-kharif	Rabi	Pre-kharif	Rabi
Trichoderma harzianum	49.20	52.19	24.92	33.11
Pseudomonas fluorescens	50.56	53.56	26.29	34.48
T. harzianum+ P. fluorescens	49.53	52.52	25.26	33.45
Hydropriming (Control)	68.23	71.22	43.73	48.70
C.D. at 5%	6.16	6.56	6.02	5.53
CV%	6.02	5.71	10.64	7.84

The values mentioned above were the mean of Percent disease index three replications *fluorescens* treated animals. Is followed by a similar tendency as cercospora leaf spot, among other things.

The control of disease by different bio-priming may be measured using the PDC (Percent Disease Control) number, which considers the control of the disease (PDI value). The numerous graphical representations (Figs. 1 and 2) revealed the various forms of PDC value to manage disease in this case. Figures 1 and 2 depicted alternative bio-priming scenarios using PDC values in two genotypes and two seasons, Masinga morok and G-4, respectively. In 35 and 60 DAT, the presentation clearly demonstrated the improved efficacy of coupled bio-priming. Over Rabi cropping, the pre-kharif season was most beneficial in both the 35 and 60 DAT phases of the plant. In both seasons, the efficiency of 35 DAT in controlling both disease was higher.

The bio-priming approach was widely used in many vegetable crops, but it was uncommon in the chilli crop, particularly in terms of disease management. However, there were other cases where bio-priming demonstrated its usefulness in disease prevention as well as other positive seedling establishing activities. Plant growth promoting bacteria

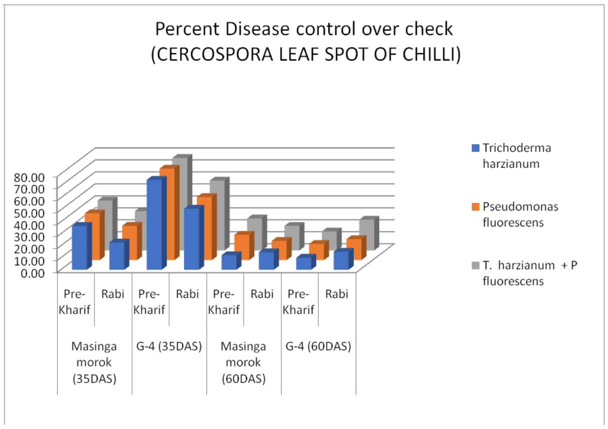

Fig. 1. Percent disease control of Seed bio-priming on Cercosporaleaf spot of chilli (cv. Masingamorok and cv. G-4) under 35 and 60 DAT of two seasons

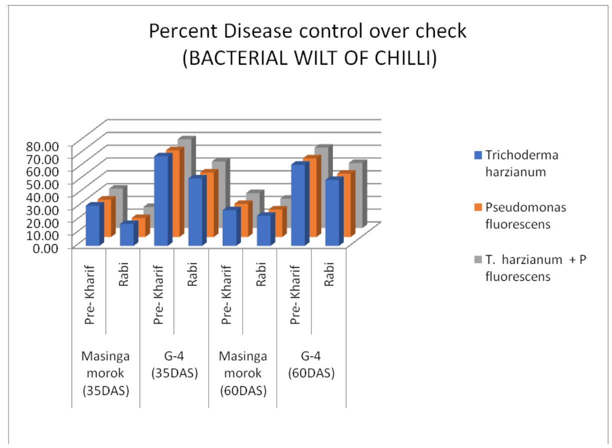

Fig. 2. Percent disease control of Seed bio-priming on Bacterial wilt of chilli (cv. Masinga morok and cv. G-4) under 35 and 60 DAT of two seasons

Table 3. Effect of bio-priming in incidence of Bacterial wilt in chilli at 35 DAT considering two seasons

Treatments	Masingamorok		G-4	
	Pre-kharif	Rabi	Pre-kharif	Rabi
Trichoderma harzianum	50.73	61.77	19.90	29.40
Pseudomonas fluorescens	48.77	57.10	18.90	28.40
T. harzianum+ P. fluorescens	46.70	53.73	18.23	25.03
Hydropriming (Control)	80.00	90.51	22.80	35.63
C.D. at 5%	6.93	9.08	6.02	4.11
CV%	7.50	7.93	10.64	7.37

Table 4. Effect of bio-priming in incidence of Bacterial wilt in chilli at 60 DAT considering two seasons

Treatments	Masingamorok		G-4	
	Pre-kharif	Rabi	Pre-kharif	Rabi
Trichoderma harzianum	52.43	63.50	25.90	35.05
Pseudomonas fluorescens	47.10	62.50	24.90	34.05
T. harzianum + P. fluorescens	43.73	59.13	24.23	30.68
Hydropriming (Control)	59.67	74.40	28.80	41.28
C.D. at 5%	6.19	7.71	4.53	4.11
CV%	6.48	6.31	9.27	6.19

(PGPB) and plant growth promoting fungus (PGPF) in combination lowered disease occurrences while also improving seedling stability, uniformity, and quality (Alam *et al*., 2014, Srivastava *et al*., 2012, Yadav *et al*., 2021; Deshmukh and Sabalpara, 2019). The relevance of priming with bio-inoculants like *T. harzianum* and *P. fluorescens* may be explained by the fact that the rhizospheric and phyllospheric micro-organisms (PGPB and PGPF) were involved in vegetative growth and development as well as pathogen invasion via soil and foliar assault. These PGPB and PGPF are well-known for inhibiting phyto-pathogen entry by reinforcing the mechanical tissue through stable cell wall construction, cellulose and lignin deposition, encouraging the production of defensive enzymes and ROS molecules, and finally leading to systemic resistance (Chakraborty *et al*., 2019). Seed priming ensures that bio-agents colonise the seeds properly (khan, 1992). However, the production of metabolites such as siderophore (a source of providing iron) and chitinase (a source of providing protection against fungi) by *P. fluorescens* BAM-4 (Minaxi and Saxena, 2010) was also observed. In the treatment of seed-borne and soil-borne diseases, they were responsible for antibiosis, establishing systemic resistance in plants, and resisting pathogen assault. Enhancing the manufacture of phenolic compounds, defence and anti-oxidative enzymes, and lowering lesion growth and reactive oxygen species buildup in chilli resulted in induced systemic resistance (Yadav *et al*., 2021). In the presence of *T. harzianum* and other similar microorganisms, the generation of ROS molecules led to the synthesis of various defence enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and anti-oxidative enzymes such as catalase, super-oxide dismutase, guaiacol peroxidase, and anti-oxidative enzymes such as catalase, super (Yadav *et al*., 2021)

Conclusion and future scope

The bio-priming technology was a novel innovation that served as bio-control agents as well as enhancing seed vigour, particularly at the seedling establishment stage. The application of several microorganisms (PGPB and PGPF) to a specific crop might help manage disease while improving productivity and quality, especially in seed production. The usage of a combination of microorganisms (*T. harzianum* and *P. fluorescens*) will assist to avoid both cercospora leaf spot and bacterial wilt while also providing healthy seedlings. T3 (*T. harzianum* + P. fluorescens) has been shown to be a better bio-control agent in the management of cercospora leaf spot and bacterial wilt leaf spot in chillies than other seed treating agents. These bacteria can be used as bio-fertilizers and bio-pesticides that are less harmful to the environment. The BCAs have the ability to create secondary metabolites and enzymes that prevent pathogens from penetrating, multiplying, and establishing themselves inside plants (Williams *et al*., 1996). The present investigation is fruitful in fruit/seed production of chilli though an attention must be needed for cultivation season as well as genotype nature.

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Conflict of Interest

There is no conflict of interests to declare to publish this article.

References

Alam, M.Z., Hamim, I., Ali M.A. and Ashrafuzzaman, M. 2014. Effect of Seed Treatment on Seedling Health of Chilli. *J. Environ. Sci. & Natural Resources.* 7(1): 177-181.

Burr, T.J. and Caesar, A.J. 1984. Beneficial plant bacteria. CRC *Crit Rev Plant Sci*. 2:1–20.

Chakraborty, N., Mukherjee, K., Sarkar, A., Acharya, K. 2019. Interaction between Bean and *Colletotrichum gloeosporioides*: Understanding through a biochemical approach. *Plants.* 8: 345.

Denny, T.P. 2006. Plant pathogenic Ralstonia species in Plant-Associated Bacteria ed. 4 Gnanamanickam S. S., editor. (Dordrecht: Springer). 573–644.

Deshmukh, A.J. and Sabalpara, A.N. 2019. Field effect of seed biopriming on *Alternaria* leaf spot of Green gram. *The Pharma Innovation Journal.* 8(6): 195-199.

Entesari, M., Sharifzadeh, F. and Ahmadzadeh, M. 2013. Seed biopriming with *Trichoderma* species and *Pseudomonas fluorescent* on growth parameters, enzymes activity and nutritional status of soybean. *Int J Agron Plant Prod*. 4 : 610–619.

FAO, 2013. Food and Agriculture Statistics. Available from: http:// www.faostat.fao.org.

Gowtham, H., Murali, M., Singh, S.B., Lakshmeesha, T., Murthy, K.N., Amruthesh, K. and Niranjana, S. 2018. Plant growth promoting rhizobacteria- *Bacil-*

lus amyloliquefaciens improves plant growth and induces resistance in chilli against anthracnose disease. *Biol. Control.* 126: 209–217.

Islam, S., Akanda, A.M., Prova, A. and Islam, M.T., Hossain, M.M. 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Microbiol*. 6: 1360.

Jain, A., Singh, S. and Sarma, B.K. 2012. Microbial consortium–mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. *J Appl Microbiol*. 112(3): 537–550.

Keswani, C., Mishra, S. and Sarma, B.K. 2014. Unravelling the efficient applications of secondary metabolites of various *Trichoderma* spp. *Appl Microbiol Biotechnol*. 98(2): 533-544.

Khan, A.A. 1992. Preplant physiological seed conditioning. *Hort. Rev.* 3 : 131-181.

Labuschagne, N., Labuschagne, N., Pretorius, T., Pretorius, T., Idris, A.H. and Idris, A.H. 2010. Plant Growth Promoting Rhizobacteria as Biocontrol Agents Against Soil-Borne Plant Diseases. In Beneficial Microorganisms in Food and Nutraceuticals; Springer Science and Business Media LLC: Berlin, Germany, pp. 211–230.

Minaxi, Saxena J. 2010b. Characterization of *Pseudomonas aeruginosa* RM-3 as a potential Biocontrol agent. *Mycopathologia.* 170(3): 181-193.

Minaxi, Saxena J. 2010a. Disease suppression and crop improvement in moong beans (*Vigna radiata*) through *Pseudomonas and Burkholderia* strains isolated from semi -arid region of Rajasthan, India. *Biocontrol.* 55(6): 799-810.

Saxena, A., Mishra, S., Ray, S., Raghuwanshi, R. and Singh, H.B. 2020. Differential Reprogramming of Defense Network in *Capsicum annum* L. Plants Against *Colletotrichum truncatum*Infection by Phyllospheric and Rhizospheric *Trichoderma* Strains. *J. Plant Growth Regul*. 39: 751–763.

Singh, A., Jain, A. and Sarma, B.K. 2014. Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during *Sclerotium rolfsii* infection. *Microbiol Res.* 169 : 353-360.

Singh, B.N., Singh, A. and Singh, S.P. 2011. *Trichoderma harzianum* -mediated reprogramming of oxidative stress response in root apoplast of sunfl ower enhances defence against *Rhizoctonia solani* . *Eur J Plant Pathol*. 131 : 121-134.

Srivastava, A.K., Singh, R.N., Kumar, S., Kashyap, P.L. and Arora, D.K. 2012. Growth promotion and management of Alternaria leaf spot in chilli by *Richoderma harzianum. Inernational Journal of Innovative Horticulture*. 1(2): 158-163.

Williams, G. and Asher, M. 1996. Selection of rhizobacteria for the control of *Pythium ultimum* and *Aphanomyces cochlioides* on sugar-beet seedlings. *Crop. Prot*. 15: 479–486.

Yadav, M., Dubey, M.K. and Upadhyay, R.S. 2021. Systemic Resistance in Chilli Pepper against Anthracnose (Caused by *Colletotrichum truncatum*) Induced by *Trichoderma harzianum*, *Trichoderma asperellum* and *Paenibacillus dendritiformis*. *J. Fungi*. 7: 307. https://doi.org/10.3390/jof7040307