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Efficacy of Seed Bio-priming to control Alternaria leaf spot in Chilli (*Capsicum annuum* L.) under new alluvial zone

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Abstract
The potential biocontrol agents *Trichoderma harzianum*, and *Pseudomonas fluorescens* in alone or combination were used as seed-biopriming for reviewing their efficacy in reduction of the devastating disease, the *Alternaria* leaf spot in chilli (*Capsicum annuum*). Considering two important seasons, the Rabi season was comparatively imperative 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). 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 Alternaria leaf spot in chilli.

Keywords: Biopriming, trichoderma, pseudomonas, chilli, Alternaria

Introduction
Chilli (*Capsicum* sp.) is herbaceous flowering plant belonging to the family Solanaceae. It is originated in South and Central America where it has been cultivated for thousands of years. The crop is extensively cultivated throughout tropical Asia and equatorial America for its edible, pungent nature of fruits. It is a self-pollinated crop; however, considerable amount of cross-pollination (up to 10%) may occur due to roaming of bees, ants and thrips. India is the largest producer of dry chilli fruits, accounting for more than 43% of the world's total dry chilli production (Rai *et al.*, 2013). Chilli production worldwide in 2013 was 3.91 million hectares, with 1.78 t/ha average productivity of dry chilli fruits (FAO, 2013) [5]. The area of chilli crop in India is 309 thousand hectares with production of 3592 thousand million tons in 2017-18 (Department of Agriculture cooperation & Farmer's welfare). In India, West Bengal has an area, production and productivity with 70,000 hectares, 110 metric tons and 1.57 metric tons/ha (Ministry of Agriculture & Farmers welfare, GOI, 2016-17).
The basic target of agriculture is seed/fruit production. As a result, maintaining the quality of the seed/fruits using nature-based treatments with various benefits is critical. Among the various strategies, seed priming such as hydro priming, Osmo priming, solid matrix priming, Hormo-priming, chemo-priming, nutripriming, and biopriming that may be used to improve seed germination, seedling vigour, and create resistance to abiotic stress. In addition to these, the biopriming approach offers the benefit of biotic stress management. Biopriming is emphasised as a viable technology alternative toachieve the three-part goal *viz*. food-nutritional security, environmental stewardship/quality, and agricultural profitability in various agro-ecosystems. Furthermore, the risk to human health from indiscriminate usage, as well as concerns about the input's energy-intensive manufacture, cover the way for bio-inoculants to be used as a possible link for greater utilisation and use efficiency.
Plant growth promoting bacteria (PGPB) and plant growth promoting fungi (PGPF) are effective as biocontrol agents for the crop's seed and air-borne diseases (Ray *et al*., 2015; Chowdappa *et al*., 2013 and Islam *et al*., 2016) [3, 7, 13]. Through root colonisation, generation of siderophores and hormones, and nutrient absorption mechanisms, these beneficial bacteria and fungi play a role in plant growth and development (Gowtham *et al*., 2018) [6]. Compared to chemically treatment on plant, it showed the multiple modes of action, *viz*. antibiosis,

synthesis of essential secondary metabolites, cell wall degrading enzymes, callose and lignin deposition in 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-lasting protection against invading pathogens (Labuschagne *et al*., 2010) [9]. *Trichoderma* spp. are soil dwellers with the ability to suppress the growth and development of soil-borne pathogens through a variety of mechanisms 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) [14] were also observed

Alternaria Leaf spot, caused by *Alternaria solani* is a vital fungal disease that damages chillies from seed to seed. In many parts of the world, they are more severe as seed-borne pathogens. These infections will cause crop harm from the beginning. In later stages, pathogen harms fruits as well, resulting in lower yield and lower quality fruit and seed. Considering the situation, the present study is linked to control or reduce the severity of Alternaria leaf spot disease in chilli through application of bio-priming under observations on two dissimilar stages as well as two diverse seasons.

Material and Methods

During the years 2020 and 2021, an *in vivo* the experiment was undertaken at Teaching Farm, Mandouri, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India. The strains *Trichoderma harzianum* and *Pseudomonas fluorescens* were used in alone and combined mode with control (Hydro-priming), each of which were replicated in three times. The talc based formulations of *Trichoderma* spp. and *Pseudomonas* spp. containing 10^8 cfu/gm were used for seed bio-priming. The considerable genotypes of Chilli (*Capsicum annuum* L.) were Masinga morok (V1) and G4 (V2) which were sown in the field condition considering two seasons like pre-kharif and rabi for this observation. The healthy seeds were utilised after surface sterilisation with 0.1% $HgCl_2$. The 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).

The observation on Percent Disease Index under Alternaria leaf spot disease of chilli was recorded by selecting 5 plants/plot allowing for each replication. The 3 observations were considered on trifoliate leaves of base, middle and upper portion of the plant at 35 DAS and 60 DAS under Pre- kharif and Rabi seasons. For observations, the standard graded scale of 0-5 (Mathur *et al.*, 1972) [10] was used which was utilised for calculating the per cent disease Index by the following formula-

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

Results and Discussions

In present experiment, the Percent disease index (PDI) on *Alternaria* leaf spot of chilli was observed considering two genotypes under two seasons in 35 and 60 DAS stages. The variation was observed in genotypes, seasons as well as stages of plant growth. The data on Percent disease index (PDI) on *Alternaria* leaf spot was presented in the table -1 and table- 2 where the recorded value indicated a significant superior demarcation in application of different treatments as priming over control under both 35 DAS and 60 DAS 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.

Table 1: Effect of bio-priming in incidence of Alternaria leaf spot in chilli at 35 DAS considering two seasons.

Treatments	Masingamorok		G-4	
	Pre-kharif	Rabi	Pre-kharif	Rabi
Trichoderma harzianum	43.49	47.36	19.18	23.06
Pseudomonas fluorescens	44.19	48.06	17.21	21.09
T. harzianum + *P. fluorescens*	26.49	30.36	11.18	15.06
Hydropriming (Control)	60.19	64.06	34.88	38.76
C.D. at 5%	4.85	4.85	3.56	3.46
CV%	5.91	5.43	9.17	7.72

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

Table 2: Effect of bio-priming in incidence of Alternaria leaf spot in chilli at 60 DAS considering two seasons.

Treatments	Masingamorok		G-4	
	Pre-kharif	Rabi	Pre-kharif	Rabi
Trichoderma harzianum	45.94	49.82	22.63	26.51
Pseudomonas fluorescens	46.64	50.52	22.33	26.21
T. harzianum + *P. fluorescens*	28.94	32.82	18.02	21.90
Hydropriming (Control)	62.64	66.52	29.33	33.21
C.D. at 5%	4.45	4.65	2.29	2.39
CV%	5.59	5.16	5.27	4.51

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

From the table-1 and table-2, it was clearly observed that the more incidence of disease was followed in 60 DAS considering both genotypes and seasons. The incidence of disease reactions showed same trend where the effect of *T. harzianum* and *P. fluorescens* was more or less similar. Observing the pre-kharif and rabi result, the two genotypes showed a variation n their disease incidence pattern where the value of Masinga morok (V1) genotype exposed higher PDI highlighting its more susceptibility to disease that can be proved in both pre-kharif and rabi season.

The control of disease through various bio-priming can be judged by calculation of PDC (Percent Disease Control) value considering the control (PDI value). Here, the different graphical representation (Fig. 1, 2, 3, 4) indicated the various modes of PDC value to control disease. Fig. 1and Fig. 2 represented the scenario of different bio-priming through PDC value in 2 genotypes Masinga morok and G-4 respectively. The presentation clearly showed the superior effect of combined bio-priming in 35 and 60 DAS. Another observation in Fig. 3 showed the performance of genotypes where variation was most prominent in G-4. The genotype G-4 indicated super most effect to control disease at 35 DAS though it was not so effective at 60 DAS. Another genotype Masinga morok exposed moderate protection against this disease where its PDC value was similar in both 35 and 60

DAS. The PDC value was also utilised in Fig. 4 to highlight the comparison effect of 2 seasons considering the best bio-priming treatment (T3) to control Alternaria leaf spot. Pre-kharif season was most effective in both35 and 60 DAS stages of plant over the Rabi cultivation. In both seasons, the effectiveness was more at 35 DAS to control the disease.

The bio-priming technique was common in many vegetable crops though it was very meagre in Chilli crop especially on observation of disease control. But, there were many instances that the bio-priming showed their efficacy in control of diseases with other beneficial activities in seedling establishment. The combination of Plant growth promoting bacteria (PGPB) and plant growth promoting fungi (PGPF) reduced the disease incidences in addition to promoting seedling stability, uniformity and better quality (Alam *et al*., 2014, Srivastava *et al*., 2012, Yadav *et al*., 2021., Deshmukh AJ and Sabalpara AN, 2019) [1, 4, 15, 17]. The importance of priming with bio-inoculants such as *T. harzianum* and *P. fluorescens* may be explained by the fact that the rhizospheric and phyllospheric microorganisms (PGPB and PGPF) were engaged in vegetative growth and development, as well as reducing pathogen invasion via soil and foliar attack. These PGPB and PGPF are well-known for preventing phyto-pathogen entrance by strengthening the mechanical tissue through stable cell wall formation, cellulose and lignin deposition, promoting the synthesis of defensive enzymes and ROS molecules, and eventually leading to systemic resistance (Chakraborty *et al*., 2019) [2]. Seed priming gives proper colonization of the bio-agents to the seeds (Khan, 1992) [8]. 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) [11, 12] was also observed. These were responsible for antibiosis and in inducing the systemic resistance in plants and overcoming pathogen attack in the management of seed borne and soil borne diseases. An induced systemic resistance in chilli was also observed by enhancing the biosynthesis of phenolic compounds, defense and anti-oxidative enzymes, and reducing the lesion development and reactive oxygen species accumulation (Yadav *et al*., 2021) [17]. The observation on chilli clearly indicated the generation of ROS molecules in host-pathogen interaction lead to synthesis of various defence enzymes *viz*, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and anti-oxidative enzymes like catalase, superoxide dismutase, guaiacol peroxidase etc. in presence of *T. harzianum* and other similar microorganisms (Yadav *et al*., 2021) [17].

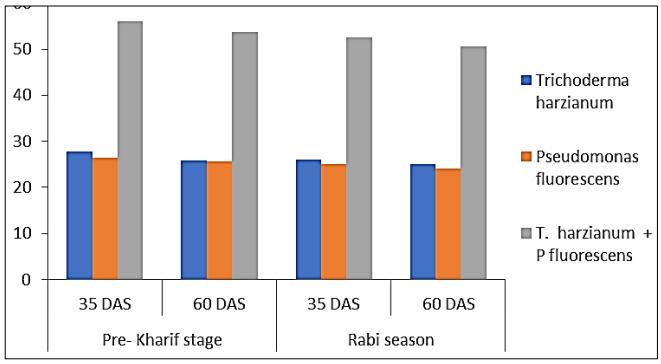

Fig 1: PDC value of Seed bio-priming on Alternaria leaf spot of chilli (cv. Masingamorok) under 35 and 60 DAS of two seasons.

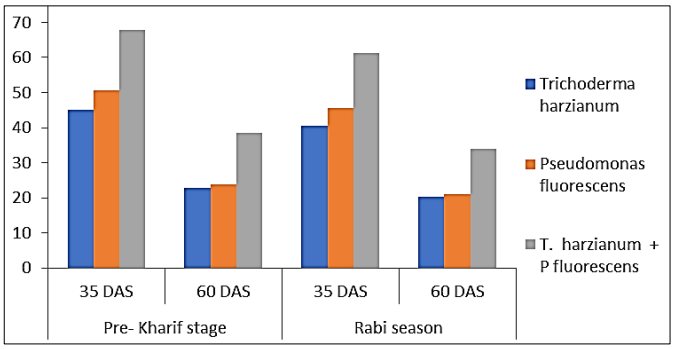

Fig 2: PDC value of Seed bio-priming on Alternaria leaf spot of chilli (cv. G-4) under 35 and 60 DAS of two seasons.

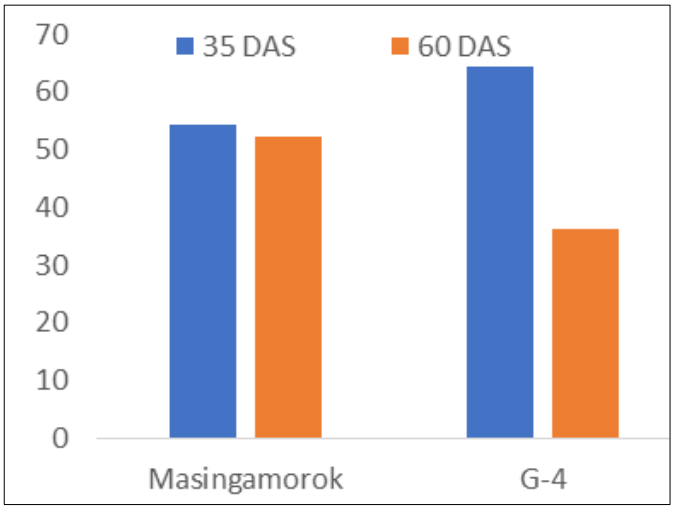

Fig 3: Comparison effect of two genotypes on PDC value considering best bio-priming (T3) to control Alternaria leaf spot.

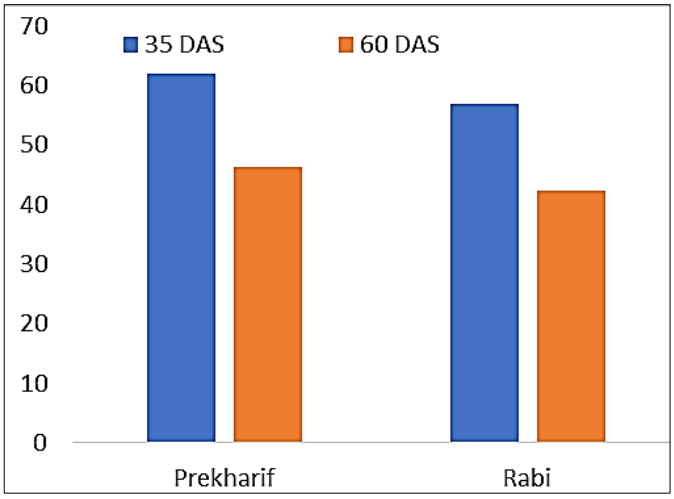

Fig 4: Comparison effect of two seasons on PDC value considering best bio-priming (T3) to control Alternaria leaf spot.

Conclusion and future scope

The bio-priming methodology was new invention as bio-control agents in addition to enhance the seed vigour particularly at initial level of seedling establishment. The proper application of various micro-organisms (PGPB and PGPF) on a particular crop can be helpful to control disease with better productivity as well as quality particularly in its seed production. The use of combined micro-organisms (*T. harzianum* and *P. fluorescens*) will be helpful to prevent the

attack of alternaria leaf spot with some additional advantage of healthy seedlings. Among different seed treating agents the treatment T3 (*T. harzianum* + *P. fluorescens*) has been proved as better bio-control agents in control of alternaria leaf spot in chilli. These bacteria are economically utilised as bio-fertilizers and bio-pesticides that are more environment friendly in the agricultural field. 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) [16]. 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.

Conflict of interest

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

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