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Studies on the effect of seed biopriming based formulations in the management of bakanae (*Fusarium moniliforme*) disease of rice

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Abstract

Rice is the most important staple food crop for millions across the world including India. There are several diseases affecting the rice crop production thereby causing great losses in the yield. Among them, rice bakanae disease is one of the serious problems especially in basmati rice growing areas like U.P, Haryana, and Punjab etc. causing yield losses up to 15% in India. *Fusarium moniliforme*, a seed and soil borne pathogen causing bakanae disease produces different kinds of symptoms like abnormal elongation of seedlings and foot rot etc. So, in the present study we conducted an experiment of seed biopriming by preparing *Trichoderma* based formulations which include different ingredients viz. Multani soil, Molasses, NPK fertilizer, Neem cake, Propiconazole in total seven different treatment combinations and one untreated control. Results from our study proved that out of these seven different treatment combinations, The minimum disease incidence of 8.93 and 14.48% were recorded in Multani soil + *Trichoderma* + Propiconazole + Molasses and maximum disease incidence 26.85 and 27.14% were recorded in Multani soil + *Trichoderma* at 15 and 30 DAS respectively. The maximum plant height of 10.03 and 17.43 cm were recorded in the case of Multani soil + *Trichoderma* + Molasses combination and minimum plant height of 6.60 and 10.33 cm was recorded in case of Multani soil + *Trichoderma* + Propiconazole + Molasses at 15 and 30 DAS respectively.

Keywords: Rice, plant growth parameters, Bakanae disease, *Fusarium moniliforme*, disease incidence, management

Introduction

Rice (*Oryza sativa* L.) is the staple food crop for more than 3 billion people around the world. Realizing the global importance, rice has shaped the diets, culture, and economy of millions of people (Anonymous, 2003) [1]. Rice is the food of more than 60% of the world's population. It is the staple food of most of south-eastern Asia. About 90% of the total rice grown in the world is produced and consumed in the Asian region. Rice provides 21% of energy and 15% of the protein requirements of the human population globally (Depar, *et al.*, 2011) [5]. Two major rice species *Oryza sativa indica* and *Oryza sativa japonica* are grown worldwide. Rice belongs to family Poaceae (old Gramineae). The commonly cultivated rice is an annual crop which usually grows to the height of half-meter or two meters but some varieties grow up to 6-9 meter (Anonymous, 2006) [2]. It is grown in all continents except Antarctica and occupying about 150 mha area, producing 573 mt rice with an average productivity of 3.83 tones/ha. India is the second-largest producer and consumer of rice in the world after China. India ranks first in the world for the production and export of basmati rice. About 70% of basmati rice is produced in India, where, it shares 7.76 million hectares with a production of 35.0 lakh metric tonnes (Anonymous, 2013) [3].

In India, the area, production, and productivity of rice is 43.42 mha, 105.24 mt and 2.42 t/ha, respectively. However, Uttar Pradesh is the largest rice-growing state after West Bengal but its productivity is low. The productivity of West Bengal and UP is 2.88 and 2.13 t/ha respectively. In UP rice occupies an area of 5.86 mha, produces 14.41 mt rice with a productivity of 2.46 t/ha in UP (Anonymous, 2016) [4]. Foot rot and bakanae of rice caused by *Fusarium moniliforme* Sheldon [*Gibberella fujikuroi* (Sawada) Wollenweber] occurs in almost all the rice growing areas of the world and causes substantial yield losses.

The pathogen is primarily seed borne but also survives in soil and diseased plant debris in certain area (Ou, 1985) [8, 9]. The most striking symptom of this disease is yellowing and abnormal elongation of infected rice seedling which led to the name bakanae. The other symptoms of this disease are foot rot, seedling rot, grain sterility and grain discoloration (Ou, 1985) [8, 9]. In recent years, bakanae disease has been spreading and being reported from newer parts of Asia. In China, Japan, Thailand, Pakistan, Bangladesh and Nepal bakanae has become a major disease since last decade and 10-70 percent disease incidence was recorded in basmati and improved rice cultivars. In India, bakanae disease incidence has been increasing considerably and moderate to severe yield losses ranging from 15-25 percent have been reported from Eastern U.P., Assam, Andhra Pradesh, Tamilnadu, Haryana and Punjab (Rathaiah *et al.*, 1991, Sunder *et al.*, 1998 and Pannu *et al.*, 2012) [11, 13, 10].

Materials and Methods

Various *Trichoderma* spp based formulations were tested to check the efficacy of formulations against *Fusarium moniliforme* with the help of seed biopriming technique.

Collection of *Trichoderma*: The culture of *Trichoderma* isolate was obtained from the Nematology Laboratory, Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U.P.

Mass multiplication of *Trichoderma*

Wheat grains were used for mass multiplication of *Trichoderma* isolates. Wheat grains were soaked in water for 12 hours and then spread on paper to remove the extra water. Dextrose was added in wheat seeds @ 20gm/kg seed and then 250 gm of wheat grain were taken in each 500 ml conical flasks. Flasks with wheat grains were plugged with nonabsorbent cotton and wrapped with aluminum foil and sterilized in an autoclave at 121°C temperature at 15 lbs pressure/inch² for 15 minutes. After proper cooling of the

conical flask, containing sterilized wheat grains were inoculated with 5 mm diameter PDA discs punched from the periphery of actively growing 5 days old culture of *Trichoderma*. All inoculated conical flasks were incubated in a BOD incubator at 26±2 °C temperature. Incubated flasks were allowed to grow *Trichoderma* with periodic shaking of the flasks so that the surfaces of all wheat seeds were colonized with thick mycelial growth of *Trichoderma* properly. After full colonization of the wheat grain by *Trichoderma*, colonized grains were takeout from the flask and air-dried in shaded conditions. The sterilized dried wheat grains were ground with the help of mechanical grinder Plate No. 01.

Plate 01: Mass multiplication of *Trichoderma*

Isolation and purification of pathogen

Rice plant showing typical symptoms of bakanae disease were collected from the CRC Chirori farm of the University. The collected disease specimen brought to the laboratory and critically examined and studied for the symptoms of the disease and isolation of the pathogen. Plate No. 02

Plate 02: Purification of pathogen

Preparation of formulations: *Trichoderma* formulation was prepared by mixing different substances with *Trichoderma* wheat grain powder. The different formulation was developed by mixing all the substances in different combination viz.

Multani soil @10 gm/kg of seed, *Trichoderma*@10 gm/kg of seed, Neem cake@10 gm/kg of seed, Propiconazole@1 ml/liter of water, Molasses@10 ml/kg of seed, and NPK@10 gm/kg of seed. Plate No.03

Plate 03: Materials used at the time of experiment for the preparation of formulations

Biopriming of Seed: Biopriming of the seed of the variety PB1121 was done with the formulation of *Trichoderma*. For biopriming of seed, seeds were soaked overnight for about 12 hrs. and then treated with a slurry of bio formulation of *Trichoderma* as per treatment. Seven *Trichoderma* based formulations were prepared in which first formulation was Multani soil + *Trichoderma* + Neem Cake + Propiconazole + Molasses + NPK, second formulation Multani soil + *Trichoderma* + Propiconazole + Molasses + NPK, third formulation Multani soil + *Trichoderma* + Propiconazole + Molasses, fourth formulation Multani soil + *Trichoderma* + Molasses, fifth formulation Multani soil + *Trichoderma* + Neem Cake, sixth formulation Multani soil + *Trichoderma* and seventh formulation was Multani soil + *Trichoderma* + Propiconazole. After that, each *Trichoderma* based formulations were applied for the seed biopriming on overnight soaked rice seed. Bio primed seeds were sown in the sterilized soil filled in the plastic pots. Inoculation of *Fusarium moniliforme* culture @ 3 g/kg of soil. Control was maintained without giving any treatment. Inoculated pots were kept in natural environmental conditions. Observations recorded on plant growth parameters such as plant height, root length, and disease incidence. The percent disease incidence was calculated according to the following formula (Teng and James, 2001; Zainudin *et al*; 2008) [14, 15].

$$\text{Percent disease incidence} = \frac{\text{Total no. of infected plants}}{\text{Total plant examined}} \times 100$$

Result and Discussion

Effect of seed biopriming with different formulation on growth parameter and bakanae disease incidence of the rice plant.

Plant height: Table-1 and Plate No.04 All tested formulations significantly increased the plant height and root length of rice plant as compared to control. The maximum plant height 10.03 and 17.43 cm was recorded in case of Multani soil + *Trichoderma* + Molasses at 15 DAS and 30 DAS respectively followed by 9.33 and 16.57 in Multani soil + *Trichoderma* + Neem Cake, 9.10 and 15.83 cm in Multani soil + *Trichoderma*, and 8.0 and 10.87 cm in Multani soil + *Trichoderma* + Propiconazole at 15 DAS and 30 DAS respectively. The minimum plant height 6.60 and 10.33 cm was recorded in case of Multani soil + *Trichoderma* + Propiconazole + Molasses followed by (6.97 and 10.50 cm) in Multani soil + *Trichoderma* + Propiconazole + Molasses + NPK as compare to control 8.50 cm and 12.50 cm at 15 DAS

and 30 DAS respectively. M. Kokila and M. Bhaskaran (2016) [7] found that seed treatment for improving seedling vigor to standardize the optimum concentration of liquid *Azospirillum* for rice bio priming seed treatment and duration of bio priming. Rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R seeds were bio primed separately with 10, 15 and 20 percent of *Azospirillum* for 6, 12, 18 and 24 hrs. of durations. Unprimed and hydro primed seeds were used as control. The result revealed that rice seeds bio primed with 20 percent *Azospirillum* for 12 h was found to be the best seed bio priming treatment for both CORH 4 rice hybrid and its parental lines to enhance the germination rate, total germination percentage, seedling growth, and vigor.

Root Length

Data of Table-1 and Plate No.04 indicates that all tested formulations were significantly increased the root length of rice compared to control. The maximum root length 7.67cm was recorded in Multani soil + *Trichoderma* + Molasses followed by 7.33, 6.33, 6.33, 5.33 and 5.33 cm in Multani soil + *Trichoderma* + Neem Cake, Multani soil + *Trichoderma* + Propiconazole + Molasses, Multani soil + *Trichoderma*, Multani soil + *Trichoderma* + Propiconazole + Molasses + NPK, and Multani soil + *Trichoderma* + Propiconazole respectively. The minimum root length 5.03 cm was recorded in Multani soil + *Trichoderma* + Neem Cake + Propiconazole + Molasses + NPK. Whereas, 7.33 cm was recorded in case of control at 30 days after sowing respectively. Dezfali *et al*. (2008) [6] found that influence of seed priming techniques on germination and early growth of the seedling. Hydropriming resulted in the lower time taken to 50% germination and higher germination index, vigor index and final germination percentage in both genotypes. Maximum invigoration was observed in seeds hydro primed for 36 h as indicated by higher germination rate, radical length.

Effect on bakanae disease incidence

Table-1 and Plate No.05 indicates that all tested formulations significantly reduced the disease incidence as compared to control. The minimum 8.93 and 14.48% disease incidence was recorded in Multani soil + *Trichoderma* + Propiconazole + Molasses followed by 10.31 and 14.48%, 11.57 and 15.28%, 16.98 and 16.98%, 22.22 and 22.22%, 23.15 and 24.09% in Multani soil + *Trichoderma* + Propiconazole + Molasses + NPK, Multani soil + *Trichoderma* + Propiconazole, Multani soil + *Trichoderma* + Neem Cake + Propiconazole + Molasses + NPK, Multani soil +

Trichoderma and Multani soil + *Trichoderma* + Molasses respectively. The Maximum disease incidence 26.85 and 27.14% was recorded in Multani soil + *Trichoderma* whereas, 34.72 and 36.11% was recorded in case of control at 15 and 30 days after sowing respectively. Sandhu *et al.* (2016) [12] Bakanae disease was recorded in all the basmati growing fields and up to 10 percent incidence was recorded on different aromatic rice cultivars. Pusa Basmati 1401 was

observed more susceptible followed by Pusa Basmati 1121 and Pusa Basmati 1509. Basmati crop raised at the recommended period showed lower disease incidence than the early transplanted crop. The recommended technology to manage this disease *i.e.* seed soaking in pesticide solution containing carbendazim 50 WP @ 0.2 percent + Streptocycline 0.01 percent (one g) for 12 hrs. followed by seedling root dip in carbendazim 50 WP (0.2%) for 6 hrs.

Table 1: Effect of seed biopriming on growth parameter and bakanae disease incidence of the rice plant.

Treatment	Plant Height (cm)		Root Length (cm)	Disease Incidence (%)	
	15 DAS	30 DAS	30 DAS	15 DAS	30 DAS
T ₁ - Multani soil + <i>Trichoderma</i> + Neem Cake + Propiconazole + Molasses + NPK	7.50	10.50	5.03	16.98	16.98
T ₂ - Multani soil + <i>Trichoderma</i> + Propiconazole + Molasses + NPK	6.97	10.50	5.33	10.31	14.48
T ₃ - Multani soil + <i>Trichoderma</i> + Propiconazole + Molasses	6.60	10.33	6.33	8.93	14.48
T ₄ - Multani soil + <i>Trichoderma</i> + Molasses	10.03	17.43	7.67	23.15	24.09
T ₅ - Multani soil + <i>Trichoderma</i>	9.33	16.57	7.33	26.85	27.14
T ₆ - Multani soil	9.10	15.83	6.33	22.22	22.22
T ₇ - Multani soil + <i>Trichoderma</i> + Propiconazole	8.0	10.33	5.33	11.57	15.28
T ₈ - Control (without any treatment)	8.5	12.50	7.33	34.72	36.11
CD at 5% level	0.925	0.678	1.196	2.289	4.847

Plate 04: Effect of seed biopriming on the growth parameter of the rice plant

Plate 05: Effect of seed biopriming on the incidence of bakanae disease of the rice plant.

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