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Effect of bio-priming and fungicides on wheat seed quality and health

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ABSTRACT Wheat is a predominant cereal crop, which has come under biotic and abiotic stresses due to shift in climate regimes in last few years. It has lead to increased incidence of seed-borne diseases of minor importance like black point (*Drechslera sorokiniana*, *Alternaria* spp., *Curvularia* spp. etc.). The present studies were conducted at Seed Technology Centre of Punjab Agricultural University, Ludhiana, to explore the benefit of bio-priming to reduce seed-borne inoculums load and to enhance seed quality. Hydro-priming alone and in combination with *T. harzianum* and captaf were at par and significantly better than control, for all the seed quality parameters *i.e.* percent seed germination (84.8, 84 and 83/76), percent emergence (76, 74, 74/64), speed of germination (23, 21.7, 22/11.75), vigour index I (2306.6, 2066.4, 2224.4/1755.6) and vigour index II (15.94, 15.71, 15.27/13.22). Seed priming with tilt significantly reduced the incidence of *D. sorokiniana*, *Alternaria tenuis* and *Curvularia lunata* by 96.9%, 96.6% and 97.9%, respectively over control. It was much better than captaf and *T. harzianum* bio-priming treatments where, the corresponding figures were 66.0% and 45.4; 74.0 and 57.4 and 55.9 and 42.0 percent, respectively.However, it failed to invigorate seed due to reduced germination of 72% and vigour index I and II of 1353.6 and 11.10, respectively. Therefore, tilt may be avoided for pre-sowing seed treatments as it suppressed the growth and inhibited the germination and related seed quality parameters. Further studies are needed to establish its use, may be in different formulation, as pre-storage seed treatment in case the seed is heavily infected with black point disease.

Keywords: Bio-priming, black point, germination, hydro-priming, seed health, seed treatment

Wheat is a predominant crop, the replacement rate of which is very low as the farmers tend to use their own saved seed for 3-4 years. It is affected by number of seed-borne diseases; loose smut and flag smut being the prominent ones. With the shift in the climate pattern due to global warming, the region is experiencing comparatively warmer temperatures in February coupled with cloudy weather and rains in the month of March and April. This has resulted in aggravation of some minor diseases like black point caused by *Drechslera sorokiniana* [(Sacc.) Subram. & Jain]. Hot and humid temperatures during dough stage cause an increased incidence of black point [1]. It has not been reported to reduce the yields, but can affect the quality of grain [2]. In USA, the grading of black point seed has been standardized as U.S. no. 1 where 2% black point affected seeds are permitted and U.S. no. 2, where 4% seeds are permitted [3]. The seed carrying *Drechslera sorokiniana* is all together important, because it is also responsible for leaf blight or foliar blight [4]. Seed with symptoms of black point under storage may invite enhanced attack of storage fungi like *Aspergillus flavus*, *A. niger* etc. Field fungi and storage fungi in combination can result in significant losses in germination, viability and vigour. As seed is the basic component for achieving the desired production targets, its health and vigour is of paramount importance.

Fungicidal treatments being hazardous to human health and due to increased environmental awareness, there is need to explore alternatives to fungicidal seed treatments. Seed priming with bio-control agents has emerged as potentially effective method to reduce seed-borne diseases along with improving plant characteristics and to enhance seed quality [5]. Seed hydration is a process which results in increased

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germination rate, more uniform emergence, germination under a broader range of environments and improved seedling vigour and growth[6]. Biological seed treatment is an approach to add biological organisms to seeds to effectively control soil and seed-borne pathogens. Whereas, seed hydration in combination with biological seed treatments improve the physiological performance of the seed.

MATERIALS AND METHODS

The present investigations were carried out using three bio-control agents; two fungal (*Trichoderma harzianum*, *Trichoderma viride*, one bacterial *Pseudomonas fluorescens* each at 15g/ kg seed) and two chemicals (captaf at 3g/ kg seed and tilt at 0.2%). The talc based formulation of bio-control agents released by Department of Plant Pathology, PAU, Ludhiana, were taken for conducting the study. The experiment was carried out in completely randomized design with three replications. Revalidated seed lot of wheat cv. PBW 621 obtained from University seed farm, Ladhowal was used for giving different pre-sowing seed priming treatments. Treatments included hydration for 16-18h at room temperature, followed by air drying (hydro priming) at room temperature; priming with 0.2% solution of tilt (2ml/kg seed); hydro priming + seed treatment with captaf @ 3g/ kg seed; hydro priming + seed treatment with *T. harzianum* at 15g/kg seed; hydro priming + seed treatment with *T. viride* at 15g/kg seed;hydro priming + seed treatment with *P. fluorescens* at 15g/kg seed along with untreated seeds as control.

The observations were recorded on germination percent, speed of germination, emergence percent, vigour index I and vigour index II and seed associated mycoflora. The germination was tested with "Between the Paper" method as per ISTA [7] in quadruplicate of 100 seeds each at 25±1°C in a germinator. For determining seedling dry weight, 10 normal seedlings selected at random, were dried at 110°C for 17h and weighed. Seedling vigour index I and vigour index II were calculated as per formulae [8]. Speed of germination was computed using 50 seeds, using TP method in four replicates in Petri dishes. Daily observations of emerged seedlings were recorded till the final count day and the speed of germination was calculated [9]. For recording the mycoflora associated with different seed treatments, the blotter method [10]. The treated seeds was used from each treatment were placed in 90mm petri plate having three layers of blotter paper at the base. Twenty five seeds were kept in each Petri plate and four such plates constituted a replication. These plates were incubated at 20±1°C for 7 days in 12 hours alternate period of light and darkness. The seeds were then observed under a stereo-binocular microscope and the associated fungi were identified following the appropriate keys [11-12].

RESULTS AND DISCUSSION

The superiority of all the pre-sowing seed treatments over control was observed, except seeds treated with tilt 25EC. Of the different seed priming treatments, hydro-priming alone and in combination with *T. harzianum* and captaf were at par and significantly better than control for all the seed quality parameters (Table 1 & 2).

Comparing means among treatment revealed that combination of priming with different bio-control agents and captaf had significantly better effect on germination. Enhanced seed germination to the tune of 84.8, 84.0 and 83.0% was obtained in case of hydro-priming and its combination with *T. harzianum* and captaf respectively, as compared to 76% in control. Similar results were obtained in case of seedling emergence (76, 74, 74% as compared to 64% in control). Various workers reported improvement in germination percent, emergence and seedling

Table 1. Seed quality parameters as influenced by different seed priming treatments

Treatment	Germination (%)	Seedling Dry weight(g)	Speed of germination	Emergence (%)	#VII	#VIII
Hydro-priming	84.8[a]	0.188	23.0[a]	76[a]	2306.6[a]	15.94[a]
Hydration with Tilt 25EC	72.0[d]	0.154	17.4[b]	62[d]	1353.6[d]	11.10[c]
Hydro-priming + Captaf	83.0[a]	0.184	22.0[a]	74[a]	2224.4[a]	15.27[a]
Hydro-priming + *Trichoderma harzianum*	84.0[a]	0.187	21.7[a]	74[a]	2066.4[a]	15.71[a]
Hydro-priming + *Trichoderma viride*	80.0[b]	0.171	20.0[a]	71[b]	1664.0[c]	13.68[b]
Hydro-priming + *Pseudomonas fluorescens*	80.0[b]	0.168	18.8[b]	70[b]	1624.0[c]	13.44[b]
Control	76.0[c]	0.174	11.8[c]	64[c]	1755.6[b]	13.22[b]
CD (p=0.05)	3.86	0.01	3.2	4.44	292.89	1.44

Vigour Index

Table 2. Effect of different seed priming treatments on the recovery of field and storage fungi in wheat seed

	Drechslera-orokininana	*Alternaria-tenuis**	*Curvulari-alunata*	*Aspergi-llus flavus*	*Aspergi-llus flavus*	*Rhizopus* sp.
Hydro-priming	10.0 (3.2)[d]	49.0(44.4)[d]	12.3 (3.6)[c]	7.7 (2.9)[d]	3.3 (2.0)[c]	7.7 (2.9)[d]
Hydration with Tilt 25EC	0.3 (1.1)[a]	1.7 (5.7)[a]	0.3 (1.1)[a]	0.3 (1.1)[a]	0.0 (1.0)[a]	0.7 (1.2)[a]
Hydro-priming + Captaf	3.3 (2.0)[b]	13.0 (21.0)[b]	6.3 (2.6)[b]	2.0 (1.7)[b]	1.0 (1.3)[b]	1.7 (1.6)[b]
Hydro-priming + *Trichoderma harzianum*	5.3 (2.5)[c]	21.3 (27.4)[b]	8.3 (3.0)[b]	4.0 (2.2)[c]	0.3 (1.1)[a]	1.3 (1.5)[b]
Hydro-priming + *Trichoderma viride*	7.0 (2.8)[d]	30.7 (33.6)[c]	9.7 (3.2)[c]	1.0 (1.3)[a]	1.0 (1.3)[b]	1.3 (1.5)[b]
Hydro-priming + *Pseudomonas fluorescens*	6.7(2.7)[d]	25.3 (30.1)[c]	9.0 (3.1)[b]	2.3 (1.8)[b]	2.3 (1.8)[c]	2.0 (1.7)[c]
Control	9.7(3.2)[d]	50.0 (44.9)[d]	14.3 (3.9)[c]	7.0 (2.8)[d]	2.7 (1.9)[c]	7.3 (2.8)[d]
CD (p=0.05)	0.5	5.3	0.7	0.4	0.4	0.4

Data in parenthesis after square root transformation
*Arc sine transformed

stand by using seed priming techniques [13]. In fact, priming induces a range of biochemical changes in the seed that require initiation of the germination process *i.e.*, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation [14]. Inoculation of seeds with biological agents in combination with priming enhanced the seed quality of sunflower [15-16].

Considering speed of germination, comparison of means showed that there was significant difference between combinations of bio-priming treatments and control. The speed of germination was 23.0, 21.7, 22.0 and 20.0% in hydro-priming alone, in combination

with *Trichoderma harzianum*, captaf and *T. viride*, respectively and were at par as compared to 11.75% in control (Table 1).The reason for early emergence of the primed seed is due to the completion of pre-germination metabolic activities, making the seed ready for radical protrusion and the primed seed germinated soon after planting compared with untreated dry seed [17].

Results of vigour index I and II obtained were similar as above. There was 31.4, 17.7 and 26.7% increase over control in case of vigour index I and 20.6, 18.8 and 15.5% in case of vigour index II with hydro-priming alone and in combination with *T. harzianum* and captaf, respectively and were at par (Table 1). Results are in confirmation with earlier work of the author on soybean [18].

Seed treatments with *Trichoderma viride* and *Pseudomonas fluorescens* were next best treatments. Seed priming with tilt proved to be failure for seed quality invigoration in wheat with very poor germination (72%) and vigour index I and II of 1353.6 and 11.10, respectively. The growth indices namely micro elements absorptions and growth regulators increased significantly in bio-priming with *Trichoderma* over untreated control. Enhancement of seed inoculation with biological agents in combination with priming stabilize the efficiency of biological agents [19-21].

The untreated seed (control) was found to be contaminated with six fungi belonging to two groups, 3 each belonging to field and storage fungi *viz. Drechslera sorokiniana* (DS), *Alternaria tenuis* (AT), *Curvularia lunata* (CL) and *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* sp., respectively. *Alternaria tenuis* was the most predominant fungi with incidence of 50%, followed by *Curvularia lunata* (14.3%) and *Drechslera sorokiniana* (9.7%). Similar results were obtained by earlier workers [22-23]. These fungi are collectedly responsible for black point and leaf blight and their seed borne nature is well established [24].

Out of storage fungi, *Aspergillus flavus* and *Rhizopus* sp. had the incidence of 7.0 and 7.3% in the control, followed by *A. niger* (2.7%). *Fusarium* sp. was not detected in any treatment by the method adopted for evaluating seed health. Probably, the population of *Fusarium* spp. decreased gradually with the increase of the grain age [25].

Hydro-priming did not affect the seed associated mycoflora. In fact, it increased the incidence of *D. sorokiniana*, *A. flavus*, *A. niger* and *Rhizopus* spp. by 3, 10, 22 and 5% respectively over control. Priming with tilt 25 EC at 0.2% proved highly effective and gave significantly least incidence of all the associated fungi. It reduced the incidence of AT, CL and DS by 96.6, 97.9 and 96.9%, respectively and that of storage fungi by 90-100% over control. Priming with captaf was the next best treatment with corresponding figures of 74.0, 55.9 and 66.0% for field fungi and 63-77% for storage fungi. Out of bio-control agents, bio-priming with *T. harzianum* was better than that with *T. viride* and *P. fluorescens*. It was at par with captaf treatment for controlling *A. tenuis* and *C. lunata* with 45.4 and 57.4% reduction of incidence of fungi over control and next best to captaf in case of *Drechslera sorokiniana*, where reduction over control of 42.0% was observed (Table 2). Various workers have found the use of propiconazole and *T. harzianum* to be effective in case of leaf blight caused by *Alternaria triticina* and *Bipolaris sorokiniana*[26, 27]. Secondary metabolites of soil rhizobacteria and plant root system can enhance the availability of mineral and nutrient to the plant, improve plant nitrogen fixation ability and increase plant health by bio-control of phytopathogens [28].

Results obtained in the present study indicate that hydro-priming alone is not sufficient to control the seed associated mycoflora [29]. Therefore, it is concluded that revalidated seed of wheat can be invigorated

for better quality when hydro-primed in combination with captaf or bio-control agent *T. harizianum*. Tilt may be avoided for pre-sowing seed treatments as it suppressed the growth and inhibited the germination and related seed quality parameters. Further studies on propiconazole (tilt), may be with different formulations need to be studied as pre-storage seed treatment in case the seed is heavily infected with black point disease.

It is suggested that bio-priming is the solution to control seed-borne mycoflora and induces plant growth regulators for better establishment of crop. The findings of the present work seek to evade the application of chemicals for seed treatment.

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