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Effect of pre-storage treatment with bio-pesticides for the control of seed borne fungi in rice

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ABSTRACT: Rice is subjected to considerable number of stresses during storage. Out of ten rice cultivars, four were selected on the basis of physical purity, high germination and minimum mycoflora. The seeds of these four cultivars namely Pusa 44, PRH 10, Jaya and Pusa Sugandh 2 were treated with three plant extracts (*Piper betle, Allium sativum, Calotropis procera*) at 0.5% and 1% concentrations, one phytochemical (geraniol), two antagonists (*Kalisena* SD, *Trichoderma harzianum*) and two fungicides (vitavax 200 and carbendazim 50WP). Seed germination, seedling vigour and seed health of above said cultivars were observed at an interval of 60 days. Seed treatment with leaf extract of *Piper betle* (0.5%) increased seed germination and seedling vigour and decreased mycoflora even after six months of storage. *P. betle* 0.5% extract was found as a potent biopesticide against most of the storage and field fungi associated with rice seeds . Extract from the stem of *Calotropis procera* (1%) and Geraniol (1%) had phytotoxic property however lower concentration (0.5%) showed positive response.

Key words: Antagonist, fungicides, germination, plant extract, seed health, seedling vigour, storage

Rice is subject to a considerable number of stresses during storage. Among the biotic stresses, those most commonly encountered are rodents, insects, mites, fungi and bacteria. The common storage fungi are several species of *Aspergillus* and *Penicillium* associated with seed discoloration and deterioration during storage. As the storage period increases prevalence of field fungi (*Curvularia, Drechslera, Alternaria, Penicillium, Fusarium, Cladosporium, Trichoconis padwickii)* decreases and generally replaced by storage fungi (Ali and Deka, 1996). Use of antagonists like *Trichoderma harzianum, Aspergillus niger, Fusarium oxysporum* and *Bipolaris sorokiniana* (Manandhar *et al.*, 1998; Bora *et al.*, 1999; Kandhari *et al.*, 2000) and fungicides like dithane M-45, tricylazole, captan, benlate, bavistin (Rahman *et al.*, 2000; Krishnamurthy *et al.*, 2001) has been reported to control seed-borne fungi of rice. In view of above consideration, it was considered worth while to undertake the present study.

MATERIALS AND METHODS

Selection of cultivars: The seeds of ten popular, freshly harvested paddy cultivars (PRH 10, Jaya, Pusa Sugandh 2, Taraori Basmati, Pusa Sugandh 3, Pusa 834, Pusa 44, Pusa 677, Pusa Basmati 1, IR-64) were subjected to seed quality assessment by taking visual observation, purity analysis, moisture content, germination percentage, seedling vigour and seed health. Four cultivars (Pusa 44, PRH 10, Jaya and Pusa Sugandh 2) with maximum vigour, germination and having minimum mycofloral attack were used for further investigation.

Determination of germination percentage: The germinability of seeds was determined by using between paper method (ISTA, 2003). The germinated seeds were evaluated in to normal, abnormal seedlings and fresh ungerminated and dead seeds. Germination percentage was recorded on the basis of normal seedling only.

Determination of seedling vigour: Seedling vigour test was conducted simultaneously along with the regular germination test. Ten normal seedlings from each replicate of germinated seedlings were randomly taken after the final count of germination. Seedlings were dried overnight in an oven set at 90°C temperature and final weights

were taken. Seedlings having more dry weight were considered more vigorous.Vigour calculated by the formula (ISTA, 2003)

Vigour Index = Dry weight of ten seedlings × Germination %

For the determination of seed mycoflora standard blotter method (ISTA, 2003) was followed in order to determine the per cent incidence of different fungi associated with seeds.

Seed treatment with biopesticides and fungicides: Three plant extracts (*Piper betle, Allium sativum, Calotropis procera*) and one phytochemical (geraniol), two antagonists (*Kalisena* SD @ 8 g/kg seeds, *Trichoderma harzianum* 5354 @ 10^8 spores per ml per 10 g seeds and two systemic fungicides vitavax 200 and carbendazim 50WP (Bavistin) were used for the seed treatment of paddy cultivars.

Seed treatment: Plant extract was applied to seed at two concentration levels i.e. 0.5 % and 1 %. Stock solutions of the test extract in 12 mL of acetone (for *C. procera* and Geraniol) or distilled/ sterile water (for *P. betle* and *A. sativum*) was prepared. Fungicide vitavax 200 @ 2.5 g/kg seeds and antagonists *kalisena* SD powder (0.32 g @ 8 g/kg seeds) and spore suspension of *T. harzianum* used for seed treatment. The treated seeds were dried at 45°C overnight in a hot air chamber and kept in four compartments in 700 gauge polythene bags, which were sealed with the help of electric sealer. Seed samples were drawn at 60 days interval to undertake the following observations viz. Germination, Seedling vigour and seed health test. Polythene bags of 700 gauges were used for storage of seeds.

Preparation of plant extract: Stem pieces of *C. procera* plants, pan leaves (Bangla Pan) pieces and garlic bulbs paste were filled in individual thimble of a soxlet apparatus and extracted with freshly distilled methanol over a water bath for 2-3 days till the extraction was completed. The extract was cooled, filtered through cotton wool and the solvent was removed using rotary evaporator under reduced pressure. The solvent free amorphous extract obtained was lyophilized and kept in refrigerator for further use.

Extract of *Palmarosa citrunella* (Geraniol) was obtained from Division of Agricultural Chemicals, IARI, New Delhi.

The treated seeds were taken out at two months interval for the study of germination percentage, seedling viability, seed health.

RESULTS AND DISCUSSION

Seed germination : Initial germination of all the four cultivars were noted and it was found to be in the range of 84- 86%, highest being in case of PRH-10 while lowest in case of Pusa 44.

Pusa 44, Jaya, PRH 10 seeds treated with *C. procera* 0.5% showed 85 to 89%, after 0, 2, 4 and 6 months of storage cv. Pusa Sugandh 2 treated with *C. procera* 0.5% concentration showed 12% increase in germination after six months of storage. Seed treatment with *C. procera* 1% and Geraniol 1% showed reduced germination just after seed treatment and then it has been drastically reduced and completely inhibited the germination of seeds in all the four cultivars. In the present studies, lower doses (0.5%) of Gerniol and *C.procera* were not highly toxic though they reduced the germination but Oudhia and Tripathi (2001) reported significant effect of plant extract of *Calotropis gigantea* on germination and vigour which is contradictory to our results.

Seed treatment with aqueous leaf extract of *P. betle* (0.5%) showed increased germination over control. Continuous increase in germination was observed in Pusa Sugandh 2 and PRH 10 up to six months of storage. *P. betle* (0.5%) treated seeds showed highest germination of 94% in cv. Pusa 44, 92% in Jaya and 85.50% in cv. PRH 10 after four months of storage while 92% in Pusa Sugandh 2 was observed after six months of storage (Fig.1). Seed treatment with extract of *A. sativum* (0.5%) showed decrease in germination over a period of time during storage but there was increase in germination from 85 to 87.5 % in Jaya after 6 months of storage. Seed treatment with 1% *A. sativum* extract was also found to improve germinability. Seed treated with *T. harzianum* and *Kalisena* maintained high germination through out the period of storage. Vitavax shows highest germinability of 89%, 89.5%, 88.5% and 89.5% in Jaya, Pusa Sugandh 2, Pusa 44 and PRH10 respectively over different period of storage.

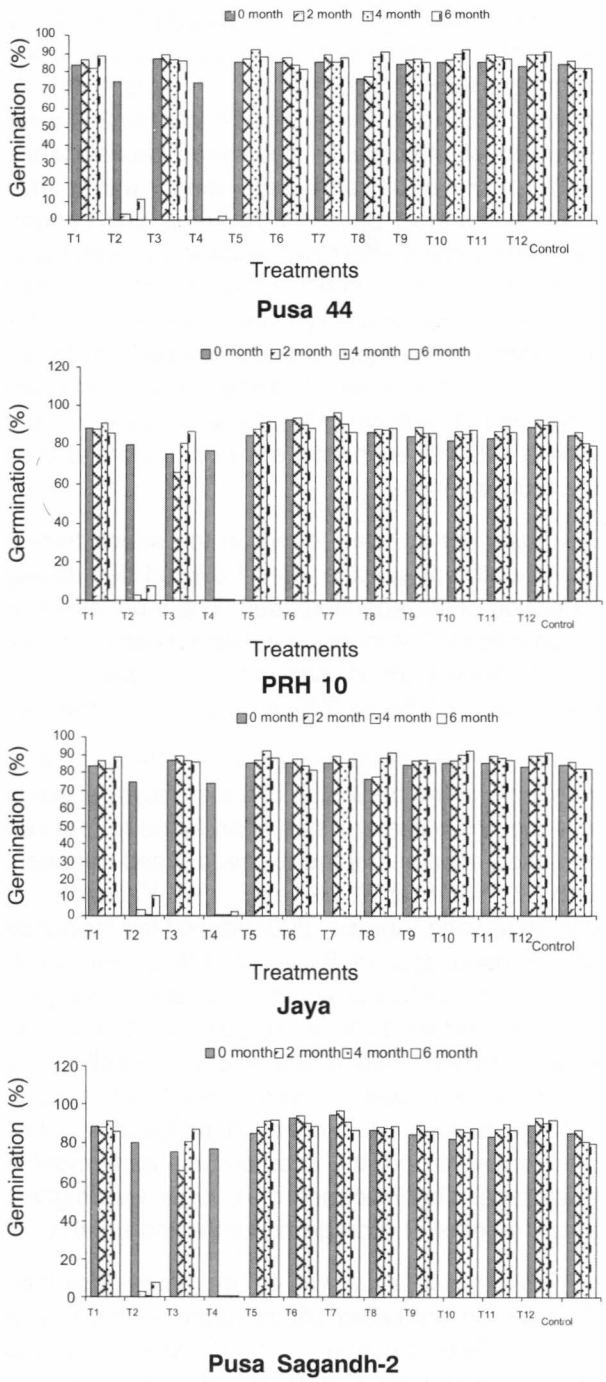

Fig. 1. Effect of seed treatments on germination of Pusa 44, PRH 10, Jaya and Pusa Sugandh-2 after different period of storage

T1=Geraniol 0.5%; T2=Geraniol 1%; *T3=Calotropis procera* 0.5%;T4=*Calotropis procera* 1%; T5=*Piper betle* 0.5%; T6=*Piper betle* 1%; T7 *Allium sativum* 0.5%; T8= *Allium sativum* 1%T9= *Trichoderma harzianum* ; T10= Kalisena; T11= Vitavax 200; T12=Bavistin

Seedling vigour

Seedling dry weight was taken after standard germination test. Seed treatment with Geraniol 0.5% and C. *procera* 0.5% showed marginal increase in dry weight in Pusa 44, PRH10, Jaya, Pusa Sugandh 2, ranging from 40 to 50 mg. Seedling dry weight of seeds treated with Geraniol 1% and *C. procera* 1% could not be recorded, as there was no germination of the seeds.

In Jaya and Pusa Sugandh 2 the seedling dry weight of the seeds treated with *P.betle* 0.5% and 1% extract increases significantly ranging from 40 mg to 80 mg after six months of storage. Seeds treated with *Piper betle* 1% extract also showed significant increase in dry weight (50 to 80 mg). Pusa 44 seeds treated with *T.harzianum* showed increased dry weight from 50 to 70 mg after six months of storage. Similar results were obtained for other cultivars. Seedling dry weight of the treated with *Kalisena* was ranging from 60 to 80 mg in Pusa 44, PRH 10, Jaya and Pusa Sugandh 2 after 180 days of storage. Seedling dry weight of the Bavistin treated seed do not showed significant increase in dry weight. Vitavax 200 is a combination of carboxin and TMTD and it has been reported that TMTD not only improved germination but also increased the seedling dry weight. Similar trend was also reported by Nakka *et al.* (1998) with three varieties of soybean treated with TMTD and stored for 120 days since vitavax-200 is a mixture of carboxin and TMTD we also got similar results when the seeds were treated with vitavax 200.

Vigour index

All the seed treatment except Geraniol and *C. procera* 1% showed increased vigour index. Seed treatment with extract of *P.betel* 0.5% conc. showed highly significant vigour index after six months of storage. The conc. of *P. betel* extract has inter-varietal response as the lower conc. of 0.5% is more effective in Jaya and PS2 while in Pusa 44 and PRH 10 higher conc. of 1% is more effective. Seed treatment with *A. sativum* extract did not show any significant difference in vigour index. Vigour index of the seeds treated by *T. harzianum* and *Kalisena* showed continuous increasing pattern in Pusa 44, PRH 10, Jaya and PS2 respectively after six months of storage. Vigour index of Pusa 44 when treated with vitavax 200 was just marginally

at par with the control and increased significantly after 4 and 6 months of storage (Table 1).

Similar trend was also reported by Nakka *et al.* (1998) with three varieties of soybean treated with TMTD and stored for 120 days.

Seed health

In the present investigation seeds of all four cultivars were found associated with *Curvularia lunata*, *Drechslera oryzae*, *D. tetramera, D. halodes, Alternaria, Fusarium moniliforme, Cladosporium cladosporioides, Trichoconis, Aspergillus flavus, A. niger* and *Penicillium*. The per cent (Angular value).The incidence of *Curvularia lunata* and *Drechslera spp* was high on untreated control after 2 months of storage.As the period of storage increased i.e. after 6 months of storage, the incidence of *Curvularia* and *Drechslera spp*. decreased in untreated control on PS2, Pusa 44, PRH 10 and Jaya respectively.

All the treatments have reduced the incidence of mycoflora effectively. Seed treatment with *P. betle* 0.5% and vitavax 200 was most effective while incidence of *Curvularia* was ranging from 4.05 to 6.80, 4.05 to 8.85 and 4.05 to 7.83 in Pusa 44, PRH 10 and Jaya respectively after 2 months of storage. In Pusa 44, *P. betle* 0.5% and vitavax 200 found to reduce incidence of *Curvularia* effectively.

After six months of storage, incidence of *Curvularia* was controlled completely by Geraniol 1% *C. procera* (0.5% and 1%), *P. betle* (0.5% and 1%), *A. sativum* (1%), vitavax 200 and bavistin. Upadhyay and Gupta (1990) reported that ethanolic extract *A. sativum* (5%) gave best inhibition of *C. lunata* while aqueous extract of 10% was found more effective against *C. lunata.*

Incidence of *Penicillium* was less before storage but it started increasing as the period of storage exceeds. In Pusa 44 and PS2 incidence of *Penicillium* was effectively reduced and eliminated by treatments with plant extract of *P. betle* (0.5%) through out the period of storage. Geraniol 1% also showed similar results and prevented *Penicillium*. Incidence of *Penicillium* on PRH 10 and Jaya was reduced effectively over the control by seed treatment with *P. betle* 0.5%, *P. betle* 1%, *T. harzianum* and vitavax 200.

Drechslera oryzae was reduced effectively by most of the treatments over the control. Seed treatment with *C. procera* 1%, *P. belte* 0.5%, *A. sativum* 0.5% and 1% along with vitavax 200 were found most effective for reducing *Dreshslera* sp. while in Pusa 44 and Jaya along with above treatment with *P. betle* 1% *Kalisena* and bavistin were also proved effective *Cladosporium* was found to be moderately infecting seeds of untreated control. *Cladosporium* sp. was found mostly after 4-6 months of storage and it was effectively controlled by seed treatment with *P. betle* 0.5%, *A. sativum* 0.5% and *A. sativum* 1%, Kalisena, bavistin and vitavax - 200 throughout the period of storage in all the four varieties.

Fusarium sp. was found on the seeds from 0 to 4 months of storage later it started decreasing as period of storage increased. The most effective treatments for *Fusarium* control were seed treatment with *P. betle* 0.5% *A. sativum* 1%, *T. harzianum,* Kalisena and Vitavax-200 in all the four varieties.

Being field fungi incidence of *Alternaria* spp. was more in the beginning of the storage period up to 4 months but later it has been found to reduced as the duration of storage exceeds. Seed treatment with *P. betle* at 0.5% and 1%, Kalisena, *T. harzianum* and Vitavax - 200 reduced the incidence of *Alternaria* spp. in Pusa 44, PS 2, and Jaya. *Trichoconis padwickii* was found very scarcely on the seed surface even in untreated control. All the treatments were found effective in controlling the incidence of *T. pawickii* during period of storage. Storage fungi predominantly *Aspergillus* sp. was nearly absent from the seed surface early months of storage. The incidence of *A. niger* was highest at six months of storage on untreated control.

Aspergillus flavus was comparatively less than *A. niger* on untreated control after six months of storage. Seed treatment with *P. betle* 0.5% and 1%, *T. harzianum*, *A. sativum* 1%, *C.procera* 1%, bavistin showed effective control against *Aspergillus* spp.

Similarly, Rahman *et al.* (2000) reported that fungi associated with rice seeds viz. *Bipolaris oryzae*, *Trichoconis padwickii*, *Curvularia lunata, Nigrospora oryzae, Alternaria tenuis, Aspergillus* spp. and *Penicillium* were found reduced following treatment with vitavax 200 with improved germination.

Table 1. Vigour index (germination × seedling dry weight) of rice seed after different period of storage

Treatment	Dose	Pusa 44				PRH 10				Jaya				PS 2			
		0 month	2 month	4 month	6 month	0 month	2 month	4 month	6 month	0 month	2 month	4 month	6 month	0 month	2 month	4 month	6 month
Geraniol	0.5%	3050	3140	3280	4220	2510	2440	4100	4250	2510	2590	4100	4420	2530	3520	5490	5130
Geraniol	1.0%	1470	0000	0000	0000	2380	0000	0000	0000	2240	0000	0000	0000	2400	0000	0000	0000
Calotropis procera	0.5%	2550	2670	4420	3440	3420	3420	0560	5160	4330	4450	5190	5980	3760	2640	4830	5220
Calotropis procera	1.0%	2210	0000	0030	0000	2450	0000	0000	0000	2230	0000	0000	0000	3080	0000	0000	0000
Piper betle	0.5%	3360	3660	4700	5250	4200	4200	5130	5370	3400	4350	5520	7040	5080	4400	5460	7360
Piper betle	1.0%	4840	5130	5400	6130	4330	4420	5400	6230	4250	4350	5880	6760	5580	3460	6300	5310
Allium sativum	0.5%	3350	3360	4520	4440	4370	4400	3440	4470	3390	3580	5950	5250	4740	4850	6330	6880
Allium sativum	1.0%	4200	4270	4650	5290	5170	4320	5370	7280	3800	3870	5280	6370	5190	3520	6130	6200
Trichoderma harzianum	10^8 spore/ml 10 g seeds	4220	4520	4980	6000	4870	4220	5280	6050	5070	5190	5100	6090	4210	4450	530	5130
Kalisena	8 g/kg seeds	5050	5010	5100	6240	5410	5400	7200	6190	4230	4320	6300	5520	4950	3480	5950	7000
Vitavax 200	2.5 g/kg seeds	3400	3540	4270	6090	3400	3460	5220	7160	5050	5340	5100	6090	4170	3480	5370	6920
Bavistin	2 g/kg seeds	3420	3520	5550	5160	4300	4350	4400	5160	4160	4300	5370	5340	4460	3720	4500	4550
Control		3360	3480	2450	2410	4320	4340	3250	3290	4210	4280	3290	3270	5070	4310	4050	3170

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