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Dravya: a Putative Organic Treatment against *Alternaria padwickii* Infection in Paddy

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Abstract: In the present study, Dravya - an organic compound used for seed treatment along with the common fungicides to test its compatibility in the management of *Alternaria padwickii* in paddy. Dravya (a sea weed extract) was found highly compatible with fungicides like Bavistin and Dithane M-45. Incidence of *Alternaria padwickii* and *Bipolaris oryzae* was also reduced to a greater extent in the paddy seed samples of Dravya treatment. On the other hand, it also enhanced the seed germination and seedling vigour. Seedlings of treated samples also showed enhanced activity of peroxidase upon challenge inoculation with *Alternaria padwickii*. The enzyme activity in the seedlings challenged with the pathogen was two fold more over control. The suppression in disease incidence in growing plants indicated the promising effect of Dravya and Dithane M-45 under green- house conditions.

Key words: Rice, *Alternaria padwickii*, stack burn, Dravya, fungicides

Rice play a paramount role in the diet of Asian countries. It is grown mainly in the irrigated areas for its nutritive values. Many hybrid varieties are released, among which high yielding varieties like IR-64, Jaya, 1001 and Mandya Vijaya are prominent in most of the fields in Karanakata. They are known to succumb to many fungal diseases like blast, brown spot, stack burn, and sheath rot. Biotic factors play a major role in breaking the resistance of the crop plants, through evolved races of soil borne and seed-borne pathogens. Fungal pathogens causes yield loss as well as reduce the nutritive quality of the grain and often leads to death of the growing plants, which are of major threat to the rice industry and hence these seed-borne diseases are of quarantine importance. India has the largest area of rice cultivation in the world of 42 M ha., and being used as staple food of 65% of the total population in India. Increase in the area of cultivation, cropping intensity, lack of effective protection measures and monoculture method of cultivation often leads to many problems related to the establishment of the diseases. Most of the present available fungicides failed to protect the crop due to adoptation of the pathogens. Hence, suitable management strategies are mandatory to overcome the problems of these pathogens. In order to combat the problem of stack burn fungus *Alternaria padwickii*, in the present study an eco-friendly approach has been employed using an organic compound, Dravya. Its compatibility was also tested with the common fungicides like Bavistin and Dithane M-45. Apart from this the compatibility of Dravya with fungicides in the induction of the defense enzymes like Peroxidases (POD) have also been evaluated with the challenge inoculation of *Alternaria padwickii*.

MATERIALS AND METHODS

Seed samples of paddy cultivar IR-64 were procured from the farmers field near K.R. Nagar, Mysore District, Karnataka State, India and were stored in polythene bags at room temperature (28 ± 2°C) till further use. From each sample the working sample was drawn after thorough mixing using a seed sampler. Among the samples, one of the sample having high incidence of *Alternaria padwickii* was selected for the entire study. In order to know the compatibility of a new phytotonic, Dravya (a sea weed extract rich in cytokinin, enzymes, biostimulants and other organic compounds such as micro and macro nutrients the product of Green life Technologies, Pvt. Ltd., Bangalore) with common synthetic fungicides, in the present study, the seeds were subjected to soaking treatment in the solution of 0.3% Dravya for 16-18 hours at 28 ± 2°C. Seeds soaked in Dravya were further air

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dried, dusted separately with Dithane M-45 and Bavistin at 0.3% concentration. 400 seeds of each treatment were plated equidistantly on three layers of wet blotters in a series of plastic plates and incubated according to the standard procedures of ISTA (Anonymous, 1993). On 8th day of incubation, the seeds were examined for the occurrence of the fungi with the aid of stereo binocular and compound microscopes and the incidence of fungi was recorded and tabulated. In the other set, seeds of similar treatment were separately plated in between wet blotter sheets, which were rolled and further, incubated for a period of 14 days under 12/12h alternate cycles of light and darkness at 22 ± 2°C. On 14th day of incubation, the seedlings were examined carefully for the essential structures. Normal seedlings were counted, the % seed germination and root- shoot length of the normal seedlings were assessed. The vigour index was calculated based on the procedures of Abdulbaki and Anderson (1974).

On the other hand, seedlings obtained from the seeds of similar treatments were subjected to enzyme assay. For this purpose, the treated seeds were sown in the wet sand beds, and then 10, 12, 14 and 16 day-old seedlings were harvested, washed and used for the extraction of the enzyme. In all the cases, seedlings raised from the untreated seeds were served as corresponding control. Further, in the other set, 8th day-old seedlings of similar treatments were challenged by spraying the spore suspension of *Alternaria padwickii* at the load of $8x10^3$ spores ml^{-1} using an atomizer. Seedlings of all the treatments, including controls were separately harvested on 10, 12, 14 and 16 days of sowing and were used for the extraction of peroxidase (POD). The protein content was estimated by dye binding method as described by Bradford (1976), using BSA (sigma) as the standard.

Enzyme extraction

1 g of freshly harvested seedlings was extracted with 0.1 M sodium phosphate buffer of pH 6.9 at 4°C, using pre-chilled mortar and pestle. The filtrate was collected and centrifuged at 10,000 rpm for 20 minutes in a refrigerated bench top centrifuge and the supernatant was collected, the same was used as enzyme source for peroxidases.

Peroxidase assay

Peroxidase (POD) activity was determined based on the procedures of Hammerschmidt et al. (1982). The reaction mixtures (3 ml) consist of 0.25% (v/v) guiacol in 10 mM H_2O_2. Addition of 20 µl of enzyme extract initiated the reaction, which was measured spectrophotometrically at 470 nm (Hitachi, 2000, Japan). For the units of POD activity, increase in the absorbance recorded at OD value at A 470/min was considered. The peroxidase activity was expressed as change in A 470/Min^{-1} and the units of activity with the mg/ protein.

RESULTS AND DISCUSSION

Table 1 provides the data relevant to the compatibility of Dravya with commonly used fungicides. Dravya in combination with Bavistin and Dithane M-45 played a major role in controlling the mycoflora. Comparative analysis of the data indicated the more compatibility of Dravya with Dithane M-45. Dravya is two fold more effective than bavistin in controlling *Alternaria padwackii* and it is equally effective as Dithane M- 45. Gradual reduction of *Alternaria padwickii* and *Bipolaris oryzae* was also observed with Dravya and Dithane M- 45 at 0.3%. Each treatment resulted in the reduced incidence of mycoflora by ten times compared to that of control. Correspondingly, increased seed germination and seedling vigour was also noticed (Table 2). Dravya alone increased the germination by 5% over the control and also the 2% increase against the Bavistin and equal to that of Dithane M-45. Dravya is more beneficial than the fungicides however, seeds treated with Dravya and Dithane M-45, resulted in the increased germination by 15% (Table 2). Thus, Dravya proved its efficiency as a promising phytotonic in enhancing the seed germination and vigour.

Table 1. Evaluation of Dravya and fungicides in relation to the incidence of seed mycoflora of paddy cv. IR-64

Treatment*	Per cent occurrence of fungi					
	Alternaria padwickii	*Bipolaris oryzae*	*Curvularia lunata*	*Trichothecium roseum*	*Verticillium cinnabarinum*	*Microdochium oryzae*
Control	15	46	2	2	1	-
Dravya (0.3%)	10	33	2	1		-
Bavistin (0.3%)	12	30	2	1		1
Dithane M-45 (0.3%)	10	16	1	1		1
Dravya + Bavistin (0.3%)	5	23	-	-	-	1
Dravya + Dithane M-45 (0.3%)	5	2	-	-	-	-

Data based on 400 seeds
Contro Seeds not treated with Dravya/Bavistin/Dithane M-45.
*Seeds were soaked in the solution of Dravya, where as Bavistin and Dithane M-45 were used as dust at 0.3%.

Table 2. Synergistic effect of Dravya and fungicides on seed germination and seedling vigour of paddy (cv. IR-64)

Treatment	Seed germination (%)	MRL ± S.E (cm)	MSL ± S.E (cm)	Vigour Index
Control	72	5.9 ± 0.06	6.1 ± 0.07	864
Dravya (0.3%)	77	6.8 ± 0.08	7.1 ± 0.06	1070
Bavistin (0.3%)	75	6.6 ± 0.86	6.9 ± 0.76	1012
Dithane M-45 (0.3%)	77	6.7 ± 0.86	7.1 ± 0.35	1062
Dravya + Bavistin (0.3%)	82	7.0 ± 0.08	7.3 ± 0.11	1172
Dravya + Dithane M-45 (0.3%)	87	7.5 ± 0.05	7.7 ± 0.07	1322

Data based on the average of 400 seeds. MRL = Mean Root Length, MSL = Mean Shoot Length, SE = Standard Error.

Data also revealed the reduced incidence of stack burn disease due to Dravya and Dithane M-45 treatment (Fig. 1). As the evident, present findings showed the effectiveness of Dravya and Dithane M-45 at 0.3% against the infection of *Alternaria padwickii* through the induction of resistance in the seedlings. Peroxidase assay indicated the increased in POD activity in the seedlings inoculated with the target fungus. Two-day seedlings showed highest activity and gradual persistence of resistance (Fig. 2). Dravya alone was effective by nine fold, where as the same in combination with Dithane M-45 was twelve fold more over the control. This shows the induction of resistance constitutively against the pathogen attack. The inducers are known to accumulate as signaling molecules mediating SAR, leading to increased expression of defense genes encoding various defense and other enzymes involved in the synthesis of phytoalexins (Vanpeer et al., 1991; M'Piga et al., 1997). Among defense enzymes, peroxidases is a constitutive enzyme, its levels are strongly modulate in response to both biotic and abiotic factors and class III plant peroxidases are believed to function in diverse physiological process including disease resistance and wound response. The POD activities that is necessary for self-defense in plant tissues against environmental stress including pathogen infection play a major role in the induction of resistance to pathogens through lignification and suberization, cross-linking of cell wall proteins, xylem wall thickening, generation of reactive oxygen species and phytoalexins synthesis (Quiroga et al., 2000; Bradely et al., 1992; Hailaire et al., 2001; Bolwell et al., 1995; Kristensen et al., 1999). Several fungicides used to manage the seed-borne diseases are known to cause adverse effects on the environment. So, in this study, Dravya was used along with Dithane M- 45 as inducer of peroxidases. There are many reports indicated the use of salicylic acid, cerobrosides as inducers of resistance against fungal diseases (Meena et al., 2001; Deepak et al., 2003). Similary, is also reported Probenazole (PBZ: 3-allyloxy-1, 2-benzisothiazlole- 1,1-dioxide) as an effective inducer of resistance against the blast fungus *M. grisea* in rice plants (Sasaki et al., 2004). Dravya and Dithne M- 45 played a major role in the induction of resistance. This may be due to their antifungal property, which acts synergistically in the

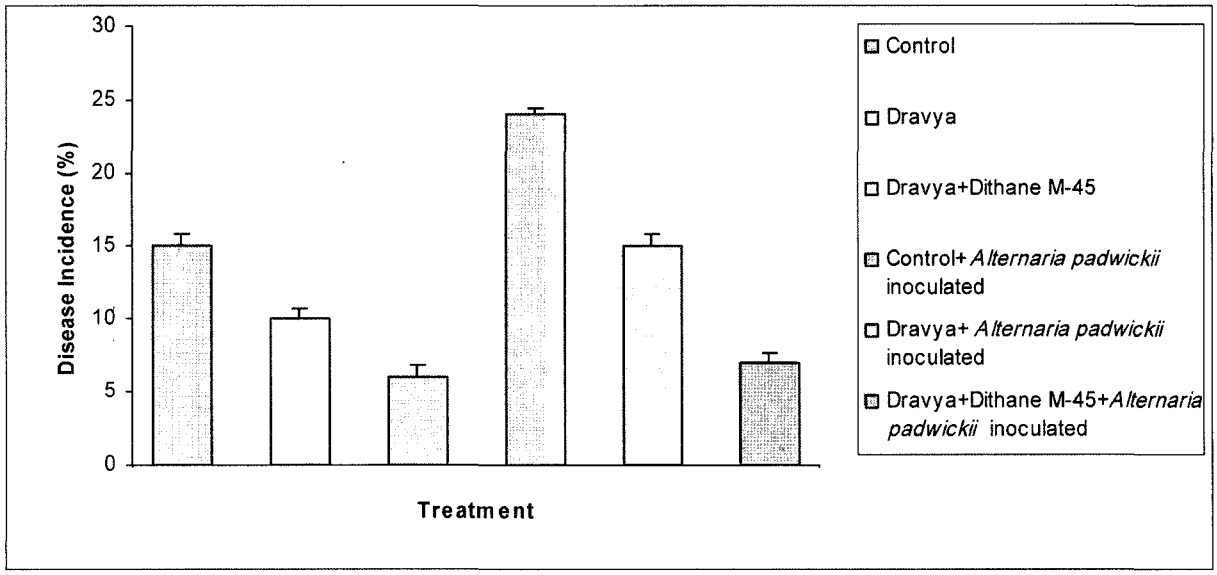

Fig. 1. Incidence of stack burn disease of paddy due to *Alternaria padwickii.*

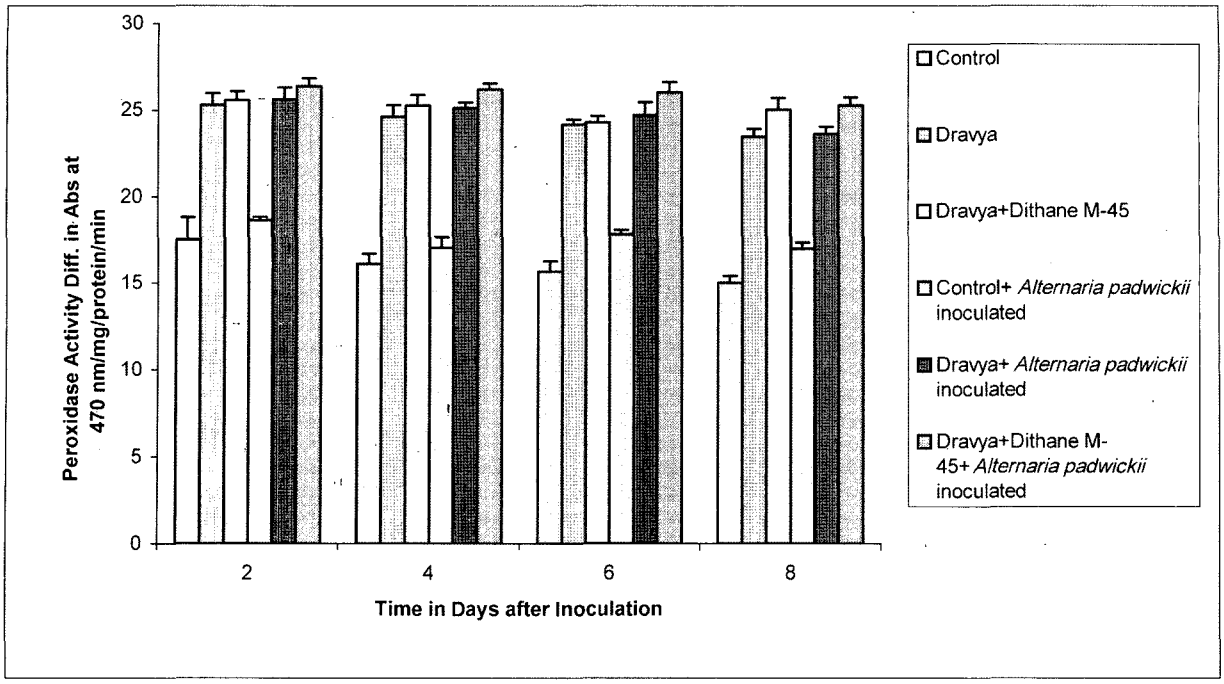

Fig. 2. Effect of Dravya and Dithane M-45 on peroxidase activity in paddy seedlings challenged with *Alternaria padwickii.*

management of fungal establishment in the host tissues. Dravya remained highly compatible with Dithane M-45, as a result there was increased seed germination, seedling vigour, through the reduce incidence of disease. Enhanced Peroxidase activity under the stress of *Alternaria padwickii* proved the potential of Dravya and Dithane M-45. Hence, dravya can be suggested for organic seed treatment against the infection of the fungal pathogens. However, it needs the repetitive experiments in field conditions in different agroclimatic zones.

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