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Dravya-A Putative Organic Treatment Against *Alternaria padwickii* Infection in Paddy

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Innate defence mechanism in plants can be triggered and enhanced by certain agents, referred as inducers against broad range of pathogens. In the present study, Dravya (a sea weed extract) was highly compatible with commonly available synthetic fungicides, Bavistin and Dithane M-45. Incidence of *Alternaria padwickii* and *Bipolaris oryzae* was also reduced to a greater extent in the paddy seed samples in Dravya treatment. Dravya 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 was two fold high after the inoculation of pathogen. The suppression in disease incidence in growing plants indicated the promising effect of Dravya and Dithane M-45 under green-house condition.

Keywords : *Alternaria padwickii*, disease suppression, Dravya, weed extract

Rice plays a paramount role in the diet of Asian countries. It is cultivated in both irrigated as well as in rain fed areas, mainly for its nutritive values. Many hybrid varieties have been released, among which high yield varieties like IR-64, Jaya, 1001 and Mandya Vijaya are prominent in Karanakata. They are known to succumb to many fungal diseases including blast, brown spot, stack burn, A biotic and biotic factors play major roles in the breaking of resistance of the crop plants, which involves soil-borne or seed-borne pathogens. Fungal pathogens causes yield loss as well as reduction of the nutritive quality of the grain and often leads to death of the growing plant. They are of major threat to the rice industry and these seed-borne diseases are of quarantine issue. India has the largest area of rice cultivation in the world of 42 M ha. Rice is, used as staple food of 65% of total population in India. Increase in the area of cultivation, cropping intensity, lack of effective protection measures and monoculture often lead to many problems related to the establishment of the diseases. Most of the fungicides available these days fail to protect the crop due to adaptation of the pathogens. Hence, suitable management strategies are mandatory to overcome the problems of these pathogens. In order to combat these problems, new eco-friendly approach for the effective management of *Alternaria padwickii, a* quarantine object in rice, a new Phytotonic, Dravya has been used along with commonly available fungicides, Bavistin and Dithane M-45. In this paper, the compatibility of Dravya with fungicides in the induction of the defense enzymes like Peroxidases (POD) have been evaluated with challenge inoculation of stack-burn causing fungus, *Alternaria padwickii.*

Materials and Methods

Seed and Seedling preparation. Paddy seed samples cv. IR-64 were procured from a farm 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, working sample was drawn with a seed sampler after thorough mixing. One of the sample with high incidence of *Alternaria padwickii* was selected for the entire study. The seeds from the selected sample were subjected to soaking treatment with Dravya. The seeds were subjected to soaking treatment in the solution of 0.3% Dravya (a product of Green life Technologies, Pvt. Ltd., Bangalore) for 16-18 hours at 26 ± 2°C. The seeds were air dried and dusted separately with Dithane M-45 or Bavistin at 0.3% concentration. Four hundred seeds of each treatment were plated equidistantly on three layers of wet blotters in a series of plastic plates and incubated according to standard procedures of ISTA (Anon., 1993). On 8^{th} day of incubation, the seeds were examined with the aid of stereo and compound microscopes for the occurrence of the fungi. The incidence of fungi was recorded and tabulated. In another set, seeds of similar treatment were separately plated in between wet blotter sheets, which were rolled and further incubated for 14 days under 12h light cycle at 22 ± 2 °C. On 14^{th} day of incubation, the seedlings were examined

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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).

Preparation of seedling for peroxidae extraction. Seedlings obtained from the seeds of similar treatments were subjected to enzyme assay. The treated seeds were sown in the wet sand beds, the 10, 12, 14 and 16 day-old seedlings were harvested, washed and used for the extraction for enzyme. In all the cases, seedlings raised from the untreated seeds were served as corresponding control.

In other set, 8^{th} day-old seedlings of similar treatments were challenged by spraying the spore suspension of *Alternaria padwickii* at the load of 8×10^3 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 for the extraction of peroxidase (POD) enzymes.

Enzyme extraction. Onegram of freshly harvested seedlings was extracted with 0.1 M sodium phosphate buffer pH 6.9 at 4°C using pre-chilled mortar and pestle. The filtrate was collected and centrifuged at 10000 rpm for 20 minutes in a refrigerated bench top centrifuge. The supernatant was collected, and was used as enzyme source for peroxidase. The protein content was estimated by dye binding method as described by Bradford (1976), using BSA (Sigma) as the standard.

Peroxidase assay. Peroxidase (POD) activity was determined based on the procedures of Hammerschmidt et al. (1982). The reaction mixtures (3 ml, should describe the details) 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 and the units of activity with the mg/protein.

Results and Discussion

Table 1 shows the compatibility of Dravya with commonly used fungicides. In combination with Bavistin and Dithane M-45 Dravya was effective was effective in controlling the mycoflora. Comparative analysis of the data indicated the more compatibility of Dravya with Dithane M-45. Gradual reduction of *Alternaria padwickii* and *Bipolaris oryzae* was also observed with 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 increase the germination by 5% over the control. However, seeds treated with Dravya and Dithane M-45, resulted in the increased germination by 15%. Thus, Dravya proved its efficiency as a promising phytotonic in enhancing the seed germination and vigour. Reduction in the incidence of stack

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

Treatment[a]	Per cent occurrence of fungi[b]					
	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		
Dravya+Bavistin (0.3%)	5	23				1
Dravya+Dithane M-45 (0.3%)	5	2	-	-		

[a]Seeds were soaked in the solution of Dravya, where as Bavistin and Dithane M-45 were used as dust at 0.3%.
[b]Data based on 400 seeds.
[c]Control = Seeds not treated with Dravya/Bavistin/Dithane M-45.

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

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
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

[a]Data based on the average of 400 seeds. MRL, Mean Root Length; MSL, Mean Shoot Length; SE, Standard Error.

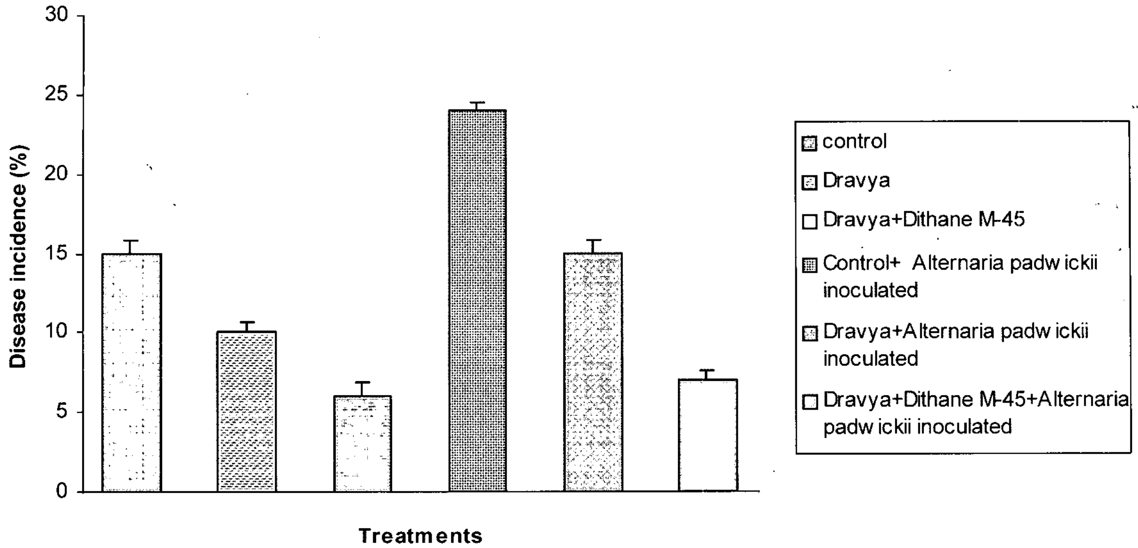

Fig. 1. Incidence of stack burn disease of paddy due to *Alternaria padwickii.*

burn disease was observed from samples treated with Dravya and Dithane M-45 in combination (Fig. 1). 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-days old seedlings showed highest activity and gradual persistence of resistance (Fig. 2). Dravya alone was effective by nine folds, where as the same in combination with Dithane M-45 was twelve folds, 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 (M'Piga et al., 1997; Van peer et al., 1991). Among defense enzymes peroxidase 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 processes including disease resistance and wound response. The POD activities that is necessary for self-defense in plant tissues against environmental stress including pathogen infection

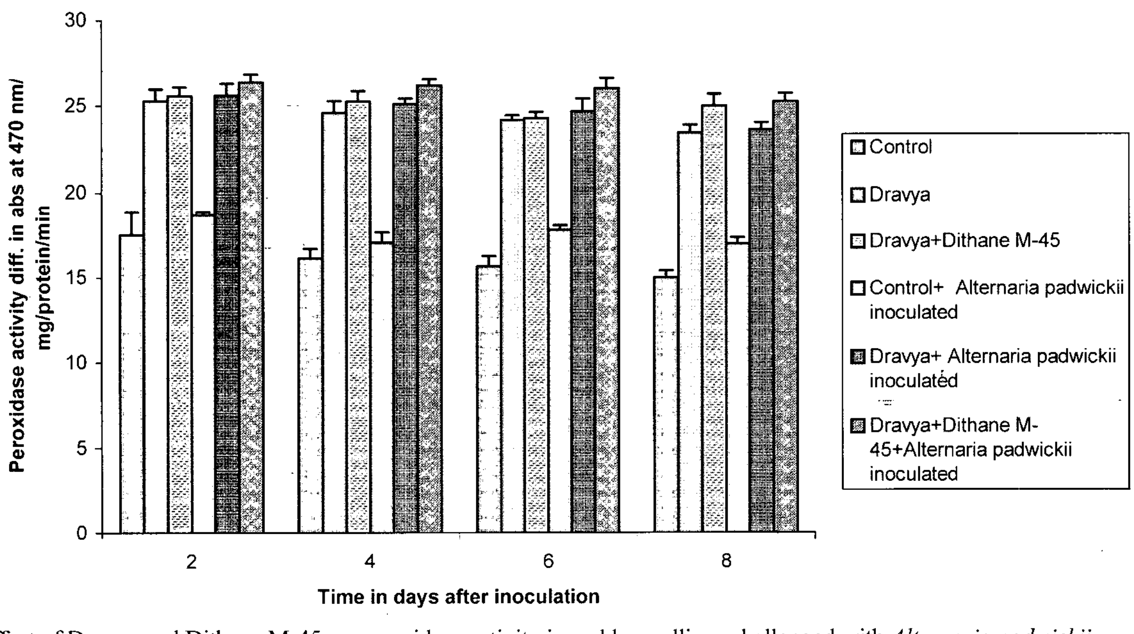

Fig. 2. Effect of Dravya and Dithane M-45 on peroxidase activity in paddy seedlings challenged with *Alternaria padwickii.*

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 (Bradely et al., 1992; Bolwell et al., 1995; Hailaire et al., 2001; Kristensen et al., 1999; Quiroga et al., 2000). Several fungicides used in the controlling of seed-borne diseases are known to cause adverse effects on the environment, ultimately domesticated animals. So, in this study, Dravya was used along with Dithane M-45 as inducer of peroxidases. There are many reports like use of salicylic acid, cerobroside as inducers of resistance against fungal diseases (Deepak et al., 2003; Meena et al., 2001). Probenazole (PBZ: 3-allyloxy-1,2-benzisothiazlole-1,1-dioxide) is also known as an effective inducers 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 that acts synergistically in the management of fungal establishment in the host tissues. Dravya remained highly compatible with Dithane M-45, as result there was increased seed germination, seedling vigour, and reduction of disease. Increased Peroxidase activity under the stress of *Alternaria padwickii* shows potential application of Dravya in combination with Dithane M-45. Further experiment, in field conditions in different agroclimatic zones will be necessary to confirm this.

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