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Role of a Phytotonic-Dravya in the Induction of Resistance of Paddy to *Bipolaris oryzae* Infection

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Abstract: In order to trigger defense mechanisms in plants, abiotic or biotic factors can be used as inducers/elicitors. In the present study, Dravya (a sea weed extract) was evaluated for its compatibility with common synthetic fungicides like Bavistin and Dithane M-45. Dravya was found to be highly compatible with Dithane M-45, in which the incidence of *Bipolaris oryzae* and *Alternaria padwickii* was reduced to a greater extent in the paddy seed sample of Cv. IR-64, a popular variety for popped rice in South India. In parallel, seed germination and seedling vigour were also enhanced over control. Treated seedlings also indicated the enhanced peroxidase and phenylalanine ammonia-lyase activities upon challenge inoculation with *Bipolaris oryzae*. Challenged seedlings showed higher activity of enzymes on second and fourth day after inoculation. The suppression in disease incidence of the seedlings was also noticed in the growing plants indicated the promising effects of Dravya with Dithane M-45 under green house conditions.

Key words: Rice, leaf spot, *Bipolaris oryzae,* Dravya, fungicides

INTRODUCTION

Rice is one of the important staple food crop, which is being cultivated in the large area for its nutritive value in India and in rest of the eastern parts of the world. Routine cultivation of the same crop leads to the gradual reduction in the yield of the crop due to various diseases outbreak as pathogen developing the resistance against exogenously applying hazardous, unsafe and narrow fungitoxic based synthetic chemicals. Among which leaf spot, blight and blast diseases are common, which takes a high toll in the field condition. In the field condition leaf spot disease often lead to nutritionally deficient quality of the crop. On contrary resistant varieties released are not stable to resist the pathogen. In this context, usage of nontoxic and environmentally safer chemicals and products to get rid of the disease is advisable. In order to overcome this problem many new resistant varieties have been released, out of which cultivars like IR-64, Jaya 1001 and Mandya Vijaya are being cultivated in major fields of South Indian states. Among these varieties, IR-64, comparatively a short duration, high yielding and disease resistant cultivar has got high demand for making popped rice. Because of high demand the farmers of Mysore district prefer this cultivar for routine cultivation. Under the sustainable cultivation of the same, the inoculum build gradually and returns to the soil every year through plant debris. As the consequence, the yield of the crop is also being reduced year by year. The application of the fungicides in terms of seed treatment as well as foliar spray safe guards the crop in the field and improves the grain yield. Out of the total production, 90% of rice is being consumed by Asian countries[1]. However, an annual loss up to 40% was reported due to biotic stress like insects, pests, pathogens and weeds throughout the world. So, to increase the net availability of the rice, the development of effective management strategy is necessary for the control of crop losses.

No chemicals remain highly persisted due to adoption of fungi. Hence, for betterment of the crop, there is a great need of the new chemical formulations. So, in the present study, a new phytotonic Dravya (a seaweed extract) has been used to test its compatibility with commonly used fungicides, Dithane M-45 and Bavistin. The efforts have been made to improve the seed germination and seedling vigour by reducing the incidence of the seed-borne fungi. In which the role of Dravya in the induction of defense related enzymes like Peroxidase (POD) and Phenylalanine ammonia-lyase (PAL) has been evaluated in the presence of brown spot causing fungus *Bipolaris oryzae.*

MATERIALS AND METHODS

Seed sample of rice cultivar IR-64 was collected from the farmers near K. R Nagar, Mysore district, Karnataka State, India and was stored in polyethylene bag at room

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condition (28±2°C) for further use. The seed sample was mixed thoroughly in a seed sampler and a working sample was drawn, such seeds were subjected to soaking treatment with Dravya. In order to know the compatibility of a new phytotonic Dravya (a product of Green Life Technology, Pvt. Ltd., Bangalore) with common synthetic fungicides, in the present study, the seeds were subjected to soaking for 16-18 h at 26±2°C in the solution of Dravya at 0.3% concentration and dusted with Dithane M-45 and Bavistin at 0.3% each. Seeds soaked in Dravya were further air-dried and dusted separately with Dithane M-45 and bavistin at 0.3%. 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 the standard procedures of ISTA[2]. On 8th day of incubation, the seeds were examined with the aid of stereo-binocular and compound microscopes for the occurrence of fungi and their incidence was recorded and tabulated. In the another set, seeds of similar treatment were separately plated on wet blotter sheets and were rolled. And such paper rolls were further incubated for a period of 14 days at 22±2°C under 12/12 h alternate cycles of light and darkness. On 14th day of incubation, the seedlings were examined carefully for the essential structures. The normal seedlings were counted and the % seed germination, root length and shoot length was recorded.

On the other hand, based on the efficacy of the fungicides, seeds treated with Dravya and Dithane M-45 were subjected to enzyme assay. For this purpose, the treated seeds were sown in the wet sand beds, the seedlings raised on the sand beds were harvested, washed and used for the extraction of enzymes. From the seeds soaked in water and Dravya separately. In the other set, 10, 12, 14 and 16-day-old seedlings raised from the seeds soaked in water and Dravya separately were considered as corresponding control.

Further, in the other set, 8-day-old seedlings of similar treatments were challenged by spraying the spore suspension of *Bipolaris oryzae* at the load of $5x10^3$ 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) and Phenyl Alanine ammonia-lyase (PAL) enzymes. The protein content was estimated by the dye binding method as described by Bradford[3], using BSA (sigma) as the standard.

Enzyme extraction: One gram of freshly harvested seedlings were extracted with 10 mM sodium phosphate buffer (pH 6.9) at 4°C using pre-chilled mortar and pestle. The filtrate was collected and centrifuged at 10000 rpm in a refrigerated bench top centrifuge and the supernatant was collected, used as enzyme source for Peroxidase. Similarly, 1 g of freshly harvested seedlings of each treatment were extracted with sodium borate buffer (0.1 M), pH 8.8 at 4°C. The supernatant was collected after centrifugation, used as the enzyme source for Phenylalanine ammonia-lyase.

Estimation of peroxidase and PAL activity: Peroxidase (POD) assay was determined based on the procedures of Hammerschmidt *et al.*[4]. The reaction mixture (3 mL) consists of 0.25% (v/v) guiacol in 10 mM H_2O_2. Addition of 100 µL of enzyme extract initiated the reaction, which was measured spectrophotometrically at 470 nm (Hitachi, U 2000, Japan), for the units of POD activity, defined as the increase in the absorbance recorded at OD value of A 470/min. The enzyme activity was expressed as change in A 470/min and units of activity with the mg/protein.

PAL activity was assessed as described by Lisker *et al.*[5]. The reaction mixture (3 mL) consisting of 50 mM L-Phenylalanine in 100 mM sodium borate buffer pH 8.8. The solution of 300 µL of crude extract was added to the mixture of L-Phenylalanine (1.5 mL) and 25 mM sodium borate buffer (1.2 mL) which were incubated for 30°C for 2 h and the OD was measured at 290 nm using a UV Spectrophotometer (Hitachi, U 2000, Japan). The enzyme activity was expressed in terms of µ mol t-cinnamic acid mg of protein/hour.

RESULTS AND DISCUSSION

Table 1 indicated the antifungal property of Dravya, which was found highly compatible with that of Dithane M-45 than Bavistin. Seed-borne fungi like *Alternaria padwickii* and *Bipolaris oryzae* showed gradual decline in their incidence compared to control. Though Dravya treated seeds showed reduced incidence of fungi, it was found highly effective with Dithane M- 45 in which seeds soaked in Dravya followed by dust treatment with Dithane M-45 at 0.3%, each treatment showed drastic reduction in the incidence of both *Alternaria padwickii and Bipolaris oryzae*. In this treatment both the fungi were found to be depleted, eight times more over control. Corresponding to the drastic reduction with incidence of fungi there was enhanced seed germination with increased vigour (Table 2). Dravya also enhanced the germination over control by 5%, however, seed treatment with Dravya followed by Dithane M-45 resulted in 18% enhancement in germination. The root-shoot length was also slightly enhanced over control. As the result, the seedlings of Dravya and Dithane M-45 treatment showed one and half a fold

Table 1: Evaluation of Dravya (a phytotonic) and its combination with fungicides in relation to seed mycoflora of paddy Cv. IR-64

	% Occurrence of fungi					
Treatments*	*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	-	1
Dravya+Bavistin (0.3%)	5	23	-	-	-	1
Dravya+Dithane M-45 (0.3%)	5	2	-	-	-	-

Data based on the average of 400 seeds, Control = 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 (a phytotonic) and fungicides on seed germination and seedling vigour of paddy Cv. IR- 64

Treatments	Seed germination* (%)	MRL±SE (cm)	MSL±SE (cm)	Vigour index
Control	68	5.5±0.01	6.2±0.04	799
Dravya (0.3%)	73	6.9±0.07	7.0±0.29	1006
Dravya+Bavistin (0.3%)	82	7.3±0.18	7.2±0.32	1077
Dravya + Dithane M-45 (0.3%)	86	7.4±0.14	7.3±0.10	1263

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

increment in their vigour. Hence, Dravya has proved its efficacy as a promising phytotonic in enhancing the seed germination and seedling growth. Results revealed the reduced incidence of disease (brown spot) on the seedlings of Dravya and Dithane M-45 treatment, in which the untreated and *Bipolaris oryzae* inoculated seedlings showed high incidence of the disease (Fig. 1).

A variety of constitutive barrier present in plants prior to infection are collectively responsible for natural resistance of plants. Physical or chemical or biological agents induces the production or accumulation of defense components in the host or activates defense mechanism in the host. It may be regarded as induced or acquired resistance.

In many fungal diseases, host cell walls after coming in contact with the fungus, there will be production or accumulation of defense related substances, which strengthen or promote the resistance of walls to fungal invasion. Production or deposition of substances in host

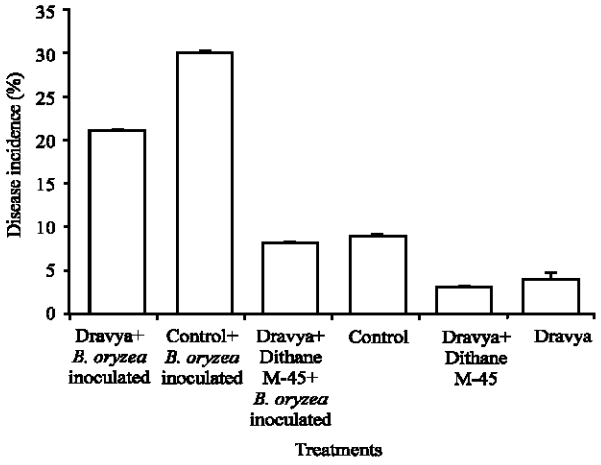

Fig. 1: Variation in the leaf spot disease of paddy due to Dravya and fungicides

cell walls include callose, glycoprotein, phenolic compounds including lignin, subrin and minerals like silica and calcium. Some of these substances are also deposited in defensive cell wall structures[6].

POD activity was comparatively found to be more in case of sample where the seedlings were challenge inoculated with *Bipolaris oryzae*. In case of seedlings un-inoculated with the fungus there was less activity irrespective of the time duration. Seedlings treated with Dravya alone showed highest POD activity on 10th day but the same remain low compared to the seedlings of Dravya and Dithane M-45 treatment. On challenge inoculation seedlings of Dravya and Dithane M-45 treatment also showed high activity of enzyme compared to their corresponding control. In all the treatment, the activity was found to be more in 10-day-old seedlings. Later on its activity was gradually declined with increased duration of time compared to control.

PAL activity was also observed to be almost same to that of POD. But, the PAL activity was more in case of 12-day-old seedlings irrespective of the treatment. Dravya alone enhanced the PAL activity over control. In case of challenge inoculation there was increased activity of the enzymes, proved the induction of resistance to *Bipolaris oryzae*. Compared to the control nearly two fold increase was observed in the treatment of Dravya and Dithane M-45. The data of Table 1 and 2 are quite comparable with that of data in Fig. 2 and 3. As the evidence of the compatibility of Dravya with Dithane M-45, there was reduction in the incidence of *Bipolaris oryzae* and in contrast the seedlings showed enhanced vigour.

This is probably the reason, which played a main role in the induction of resistance against the fungal pathogen in the seedlings through enhanced activity of POD and PAL. Present findings indicated the highly compatible nature of Dravya with Dithane M-45. The data provide the

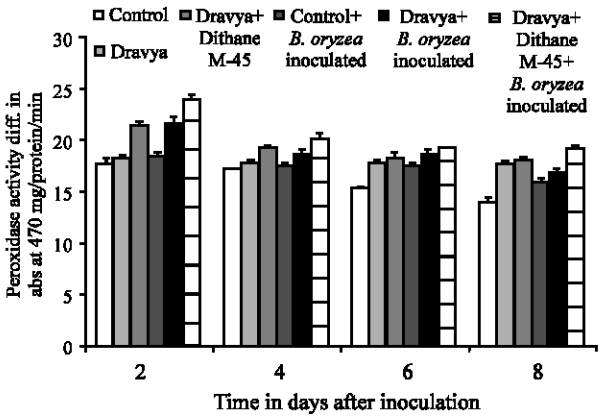

Fig. 2: Combined effect of Dravya and Dithane M-45 on the peroxidase activity in paddy seedlings

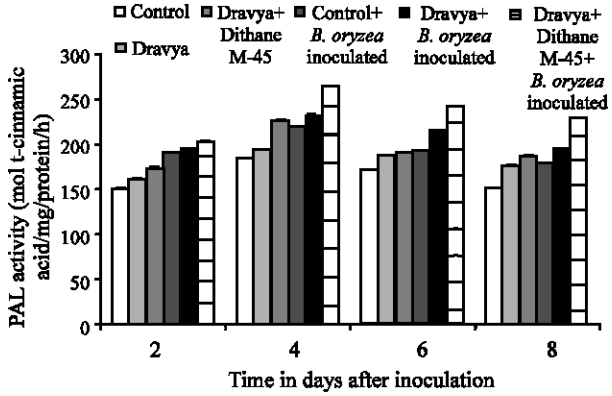

Fig. 3: Effect of Dravya and Dithane M-45 on phenylalanine ammonia-lyase in paddy seedlings

information about the close relationship of reduction in disease incidence with that of enhanced POD and PAL activity. The accumulation of POD and PAL in the host tissues are responsible for the defense against the infection of *Bipolaris oryzae*. Though *Bipolaris oryzae* is known to cause leaf and grain spots, due to its severity in epidemics, it has been considered as a quarantine object, whose germinating spores are known to produce phytotoxins like ophiobolin A and B[7]. So, in the present study, paddy sample having high infection of *Bipolaris oryzae* was considered, in which the seeds without Dravya and Dithane M-45 showed less germination, seedling vigour and high disease incidence. All these parameters are quite interrelated and were comparable with that of respective control.

Pathogen challenged cells on interaction are known to induce the accumulation of antimicrobial phytoalexins, synthesis of ethylene, deposition of lignin and other wall bound phenolic compounds and synthesis of proteins such as chitanses, wall- associated hydroxyproline-rich glycoprotein and pathogenesis-related proteins[8]. Reduction in the incidence of fungi is also due to fungicidal effect of Dithane M-45, however, the additional nutrients such as natural minerals, amino acids, algenic acid, simple and complex carbohydrates in Dravya also have played an important role in the enhancement of seedling vigour. Similar to the present observations, Meena *et al.*[9] have reported the reduction in the *Cercospora* leaf spot of groundnut upon foliar application of salicylic acid at 1 mM concentration. PAL is the enzyme of phenyal propanoid metabolism in higher plants and it has been played an important role in the accumulation of phenolics, phytolexins and lignins, which is responsible for disease resistance[10]. Induction or accumulation of the pathogenesis related proteins (Pr groups of proteins), which include the Peroxidase and β-1,3-glucanases, which are well documented in several plants after viral, bacterial and fungal infection. Peroxidase plays an important role in the biosynthesis of plant cell walls. Lignification and wall thickening are well known defense responses to pathogens particularly to fungi, another possible role for Peroxidase is the oxidative cross linking of pre existing hydroxyproline, structural proteins in the cell wall making it more resistant to degradation by microbial enzymes[11]. The antifungal activity of Dravya and Dithane M-45 might be due to the involvement in the induction of range of defense genes, which are encoded with Pathogenesis Related (PR) proteins. Bowels[12] made similar reports due to the application of salicylic acid. Dravya and Dithane M-45 might have induced chitinase and glucanases in the host tissues for which most of the substances of the cell walls of a majority of fungi remains as the substrates. This may perhaps be the probable reason for the resistance of plants to leaf spots, under the treatment of Dravya and Dithane M-45[13]. Fridlender *et al.*[14] have also proposed the similar concept with that of chitinase and β-1, 3-glucanases.

There are similar reports with respect to induction of Peroxidase activity by different biotic and abiotic stress conditions[15]. Hence, the present findings may be attributed to the same with respect to the Dravya in combination with Dithane M-45. The enhancement of seed germination and seedling growth upon treatment with Dravya in combination with Dithane M-45 is due to the suppression of fungi in the host tissues. The present results with respect to enhanced seed germination and seedling vigour are in confirmity with the findings of Deepak *et al.*[16] in which they have explained the influence of cerebroside on the stimulation of plant growth against the downy mildew pathogen in the pearl millet.

Though the compatibility of Dravya with Dithane M-45 in suppression of fungi, disease reduction and enhanced growth of seedlings under the stress of *Bipolaris oryzae* indicates its potential, it needs repetitive field trials under different agro-climatic conditions and hence, it can be popularized for commercial usage.

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