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Role of Foliar Spray in the Infection Biology and Management of Fungal Diseases of Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai]

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Abstract: Eight fungal species were found to be associated with watermelon seeds were tested for their pathogenicity under field conditions. Among which *Myrothecium verrucaria*, *Didymella bryoniae*, *Fusarium oxysporum* and *F. solani* were found to be highly pathogenic, resulted in the decreased percentage of seed germination, seedling vigour, ultimately reduced the yield of the crop. As the consequence of the severity of infection, plants showed the varied degree of leaf spots, blight, gummy stem blight and wilt symptoms. In the field to manage these diseases, the plants were separately sprayed with the fungicides at three schedules with equal intervals of 3 days. Among the fungicides, Topsin was effective against the leaf spot diseases where, as Dithane M-45 showed promising results against gummy stem blight. Bavistin efficiently controlled the wilt disease. Over all observations indicated broad-spectrum activity of Topsin in the management of fungal diseases and hence there was enhanced growth and increased yield of the crops.

Key words: Watermelon · fungal diseases · fungicides · foliar spray · fruit yield

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], an annual creeping, commercial crop grown throughout the world for its sugary, fleshy edible fruit which is eaten fresh to relieve thrust especially during hot seasons. Fruit constitutes 93-95% water, 5% carbohydrate, 0.5-1% protein and 0.2% fat, hence serves as a low calorie food for the domestic life. Fruit/juice industry in India has made a remarkable progress during the last three decades resulted in enhanced cultivation of this crop to meet its demand in the commercial market. In order to meet the commercial demand many high yielding varieties such as Arka Manik, Arka Jyoti, Durgapura Meetha, Durgapura Kesar, Pusa Bedana have been released in India. In spite of that farmers are facing severe loss of the crop due to microbial diseases in the field, which are either of seed-borne or soil borne in nature. Among microbes, fungi play a major role in bringing diseases like leaf spot, blight, gummy stem blight, wilt and fruit rot. Though many remedial measures are in practice, none of them are stable in the effective management of diseases caused by the microbes. Normally in the field disease symptoms are expressed during flowering stages. As soon as the symptoms initiates, farmers prefer the foliar sprays with the chemical fungicides. Keeping this view in mind in the present study, a popular variety has been tested in the experimental plots for its susceptibility to seed-borne fungal infection. Apart from these, commercially available chemical fungicides were also evaluated for their efficacy as foliar spray in the management of diseases caused by fungi.

MATERIALS AND METHODS

Seed sample of a popular variety of watermelon namely Arka Manik was collected from a seed trade agency at Bangalore and was stored in polyethylene bag at 28°C till further usage for the evaluation of seed mycoflora. For this purpose, 400 seeds were randomly picked and plated equidistantly on three layers of wet blotters in a series of plastic plates of 9cm diameter. Further, the plates were incubated for a period of one week under alternate cycles of near ultraviolet light (NUV) and darkness at 22 ± 2°C according to the standard procedures of ISTA [1]. On 8th day of incubation the seeds were examined stereoscopically for the occurrence of fungi. The incidence of fungi associated with the seeds was recorded and the results were tabulated.

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Fungi such as *Alternaria cucumerina* (Ellis & Everhart) Elliot, *Myrothecium verrucaria* (Albertini & Schewein) Detmar ex Fr., *Myrothecium roridum* Tode ex Fr., *Didymella bryoniae* (Awersw.) Rehm, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *Fusarium solani* (Mart.) Apple & Wollenw. emend. Snyder & Hansen, *Fusarium equiseti* (Carda) Sacc. and *Fusarium verticilloides* (Sheldon) were isolated from the incubated seeds of watermelon and their cultures were maintained on Potato Dextrose Agar plates for the purpose of seed inoculation. In order to evaluate the pathogenic effect of these species on watermelon plants, seeds of variety Arka Manik were surface sterilized with NaOCl solution of 2% available chlorine for 5 min and were washed 3-4 times with sterilized distilled water. The surface sterilized seeds were then air-dried and rolled separately on 10th day-old sporulated colonies of the above-mentioned fungi. Such inoculated seeds were sown individually in the pits measuring 1ft diameter, 0.5 ft depth made in the experimental plot and the row-to-row, plant-to-plant distance was maintained by 1 m. Each pit in the plot was supplied with basal dose of NPK (5 g pit^{-1}) and irrigated accordingly. Further, the observable parameters like seedling emergence, pre-emergence mortality and post-emergence mortality, total number of vines/plant, average length of vine/plant and total number of fruit setting/plant were recorded. Number of plants showing symptoms like leaf spot, gummy stem blight and wilt were recorded. At flowering stage, as soon as the symptom appears, the plants were sprayed separately with 3% solution of fungicides like Topsin-M 70%WP [(Dimethyl 4,4,'-o-phenylenebis (3-thioallophanate)], Dithane M-45 75%WP (Manganese ethylenebis-dithiocarbamate plus zinc), Bavistin 50%WP (Methyl-H-benzimidazole-2ylcarbamate), Captan 50%WP [N-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide], Blitox 50%WP (Copperoxychloride), Bayleton 25%WP [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1h-1,2,4-triozole-1-yl)-2-butanone 25%)] and Vitavax 50%WP (5,6-dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide) for three times at three days interval. After 10 day of fungicidal spray the total number of recovered plants were recorded. Finally the total numbers of fruits per plant were counted and the data was compiled with respect to the yield loss. Plants raised from the seeds not inoculated with any fungi were served as corresponding control. The entire data was compared with that of corresponding control.

Resultant data from repeated experiments were combined and statistics performed on the combined data were analyzed by ANOVA followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Among the fungi tested, *Myrothecium verrucaria* was found to be very aggressive, as the consequence of heavy colonization there was low percentage of healthy plants. Compared to control, all the fungal species resulted in the loss of emergence under field conditions. In case of *Myrothecium verrucaria* infection resulted in the higher rate of seed decay as well as pre-emergence death of the seedlings. *Didymella bryoniae* inoculated samples also showed higher degree of loss through the death of the seedlings before and after emergence from the soil. Where as *Fusarium verticilloides* showed least effect in which 78% of the seedlings were survived. *Fusarium solani* and *F. oxysporum* also created the stressed condition, as the consequence 10 to 15% of seedlings mortality was observed (Table 1).

In the present study, commercially available fungicides such as Topsin, Dithane M-45, Bavistin, Captan, Blitox, Bayleton and Vitavax were used for foliar spray in the field condition. After 10 days of final spray, the plants showing leaf spots, Gummy stem blight and wilt were recorded with respect to different fungi. Plants

Table 1: Effect of pathogenic fungi on the seedling emergence and mortality of watermelon (variety Arka Manik) under field conditions

Mycoflora	% Emergence ± SE	% Pre-emergence mortality ± SE	% Post- emergence mortality ± SE	% Yellowing of seedlings ± SE	% Decayed seeds ± SE	% Healthy seedlings
Alternaria cucumerina	84 ± 2.04^{b}	9 ± 0.41^{e}	5 ± 0.578^{d}	12 ± 0.82^{abc}	7 ± 0.41^{d}	67
Myrothecium verrucaria	67 ± 1.47^{d}	16 ± 0.58^{a}	11 ± 0.912^{c}	10 ± 0.58^{cd}	17 ± 1.47^{a}	30
Myrothecium roridum	71 ± 1.56^{c}	14 ± 1.47^{bc}	10 ± 1.150^{c}	13 ± 0.91^{ab}	15 ± 0.82^{ab}	48
Didymella bryoniae	75 ± 1.68^{c}	15 ± 0.91^{ab}	21 ± 1.470^{a}	14 ± 1.68^{a}	10 ± 0.41^{c}	40
Fusarium oxysporum	73 ± 2.04^{c}	12 ± 0.41^{cd}	16 ± 0.910^{b}	8 ± 0.58^{de}	15 ± 1.47^{ab}	37
Fusarium solani	76 ± 1.63^{c}	10 ± 1.40^{d}	23 ± 0.820^{a}	11 ± 0.91^{bc}	14 ± 0.58^{b}	32
Fusarium equiseti	84 ± 1.82^{b}	8 ± 0.54^{e}	16 ± 1.830^{b}	7 ± 0.81^{e}	8 ± 0.57^{cd}	53
Fusarium verticilloides	91 ± 0.89^{a}	3 ± 0.63^{f}	8 ± 0.410^{cd}	2 ± 0.41^{f}	6 ± 0.41^{d}	78
Control	94 ± 0.91^{a}	-	-	-	6 ± 0.58^{d}	94

Data were recorded on 14 day of sowing based on the average of 200 seeds of four replicates for each treatment. According to Duncan's Multiple Range Test (DMRT) the values followed by different superscripts were remain significantly different at $p \leq 0.05$, SE = Standard Error of the Mean

Table 2: Effect of foliar spray of fungicides in the management of fungal diseases of watermelon

	% incidence of disease symptoms after treatments							
	Leaf spot			Gummy stem blight			Wilt	
Treatments	*Alternaria cucumerina* ± SE	*Myrothecium verrucaria* ± SE	*Myrothecium roridum* ± SE	*Didymella bryoniae* ± SE	*Fusarium oxysporum* ± SE	*Fusarium solani* ± SE	*Fusarium equiseti* ± SE	*Fusarium verticilloides* ± SE
Topsin	7 ± 0.91^{d}	18 ± 1.93^{g}	15 ± 1.29^{e}	24 ± 0.47^{g}	22 ± 1.52^{g}	23 ± 1.29^{ef}	14 ± 0.85^{de}	8 ± 0.41^{f}
Dithane M-45	10 ± 0.41^{cd}	24 ± 1.82^{f}	17 ± 1.22^{e}	20 ± 0.91^{g}	26 ± 1.82^{f}	25 ± 1.63^{de}	15 ± 0.91^{de}	10 ± 0.91^{de}
Bavistin	13 ± 1.47^{bc}	27 ± 1.0^{ef}	21 ± 0.91^{d}	29 ± 1.29^{f}	16 ± 1.29^{h}	21 ± 1.47^{f}	10 ± 0.41^{e}	6 ± 0.58^{f}
Captan	9 ± 0.91^{d}	31 ± 2.08^{d}	25 ± 0.94^{cd}	36 ± 2.04^{e}	32 ± 0.40^{e}	28 ± 0.85^{d}	18 ± 0.85^{b}	11 ± 0.9^{cd}
Blitox	16 ± 1.29^{b}	29 ± 1.19^{de}	27 ± 1.29^{bc}	48 ± 1.47^{d}	48 ± 1.47^{d}	37 ± 1.10^{c}	24 ± 1.32^{bc}	13 ± 1.47^{bc}
Bayleton	15 ± 1.22^{b}	36 ± 1.29^{c}	27 ± 0.82^{bc}	63 ± 1.50^{b}	55 ± 0.64^{c}	54 ± 0.91^{b}	22 ± 0.84^{cd}	15 ± 0.41^{b}
Vitavax	17 ± 0.41^{b}	43 ± 0.81^{b}	29 ± 1.04^{b}	57 ± 0.91^{c}	67 ± 1.55^{b}	52 ± 0.41^{b}	25 ± 1.08^{b}	15 ± 0.48^{b}
Control + Inoculated	24 ± 1.15^{a}	66 ± 1.47^{a}	54 ± 1.08^{a}	83 ± 1.29^{a}	71 ± 1.49^{a}	82 ± 0.85^{a}	46 ± 0.64^{a}	22 ± 1.68^{a}
Control	-	-	-	-	-	-	-	-

Data based on 4 replicates

According to Duncan's Multiple Range Test (DMRT) the values followed by different superscripts were remain significantly different at $p \leq 0.05$,

SE = Standard Error of the Mean

Table 3: Efficacy of different fungicides on the growth and yield of watermelon against the infection of pathogenic fungi under field conditions

	Alternaria cucumerina			*Myrothecium verrucaria*			*Myrothecium roridum*			*Didymella bryoniae*			*Fusarium oxysporum*			*Fusarium solani*			*Fusarium verticilloides*		
Treatments	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Topsin	6^{a}	3.6^{a}	12^{a}	4^{a}	3.2^{ab}	5^{b}	4^{ab}	3.2^{b}	6^{b}	4^{ab}	3.3^{abc}	7^{b}	4^{ab}	3.3^{a}	8^{a}	4^{ab}	3.3^{ab}	5^{c}	6^{a}	3.6^{ab}	12^{ab}
Dithane M-45	5^{ab}	3.5^{ab}	10^{bc}	4^{a}	3.0^{ab}	4^{bc}	5^{a}	3.2^{b}	5^{bc}	5^{a}	3.5^{a}	9^{a}	4^{ab}	3.3^{b}	7^{b}	5^{a}	3.4^{a}	7^{b}	5^{ab}	3.5^{bc}	11^{bc}
Bavistin	4^{bc}	3.4^{abc}	9^{cd}	4^{a}	3.1^{b}	4^{c}	4^{ab}	3.1^{b}	4^{cd}	4^{ab}	3.1^{abc}	6^{b}	5^{a}	3.4^{a}	9^{a}	5^{a}	3.4^{a}	7^{b}	6^{a}	3.7^{a}	13^{a}
Captan	5^{ab}	3.4^{ab}	11^{ab}	3^{ab}	2.8^{c}	3^{c}	3^{bc}	2.8^{c}	4^{cd}	2^{c}	3.8^{cde}	3^{c}	3^{c}	3.2^{c}	5^{c}	3^{bc}	3.2^{bc}	4^{d}	5^{ab}	3.5^{bc}	9^{ed}
Blitox	4^{bc}	3.3^{bc}	7^{e}	2^{b}	2.5^{cd}	3^{c}	2^{c}	2.7^{d}	3^{d}	3^{bc}	3.2^{bcd}	3^{c}	2^{bc}	2.9^{d}	3^{d}	3^{bc}	3.0^{cd}	1^{e}	4^{bc}	3.4^{cd}	10^{cd}
Bayleton	4^{bc}	3.3^{bc}	8de	3^{ab}	2.6^{cd}	3^{c}	3^{bc}	2.8^{c}	4^{cd}	2^{c}	2.7^{bcd}	1^{d}	3^{bc}	3.0^{cd}	4^{a}	2^{cd}	2.9^{de}	1^{e}	5^{ab}	3.4^{cd}	8de
Vitavax	3^{c}	3.2^{cd}	7^{e}	2^{b}	2.5^{cd}	3^{c}	2^{c}	2.5^{d}	4^{cd}	2^{c}	3.0^{ed}	2^{cd}	2^{c}	2.7^{e}	1^{e}	1^{d}	2.8^{d}	1^{e}	4^{bc}	3.3^{de}	7^{fg}
Control + Inoculated	3^{c}	3.1^{d}	7^{e}	2^{b}	2.4^{d}	3^{c}	2^{c}	2.5^{d}	4^{cd}	2^{c}	2.8^{e}	2^{cd}	2^{c}	2.7^{e}	1^{e}	1^{d}	2.6^{f}	0^{e}	3^{c}	3.2^{de}	6^{g}
Control	4^{bc}	3.4^{ab}	8^{cd}	4^{a}	3.4^{a}	8^{a}	4^{ab}	3.4^{a}	8^{a}	4^{ab}	3.4^{ab}	8^{a}	4^{ab}	3.4^{a}	8^{a}	4^{ab}	3.4^{b}	8^{a}	4^{bc}	3.4^{cd}	8^{def}

A-number of vine/plants, B-average vine length (m/plant), C-mean total of healthy fruits/plant

Data based on 4 replicate

According to Duncan's Multiple Range Test (DMRT) the values followed by different superscripts were remain significantly different at $p \leq 0.05$

raised out of *A. cucumerina*, *M. verrucaria* and *M. roridum* inoculated seeds showed the leaf spot ranged from 24-66%. Plant raised from the seeds with *Didymella bryoniae* inoculation resulted in 83% gummy stem blight. In the similar manner *Fusarium oxysporum*, *F. solani*, *F. equiseti* and *F. verticilloides* inoculation showed the wilt symptoms at the flowering and post flowering stages, in which *F. solani* and *F. oxysporum* were found to be most aggressive (Table 2). Irrespective of fungi and treatment there was no disease incidence over control. With respect to leaf spot diseases, Topsin was found to be the best and proved its effectiveness over control. Though, Vitavax treatment reduced the leaf spot disease to some extent it was comparatively least efficient. In case of gummy stem blight, Dithane M-45 treatment was proved its efficacy over any other treatment. Plants sprayed with Bavistin showed least incidence of wilt diseases, hence it proved its efficacy against *Fusarium* species. Compared to these, Bayleton and Vitavax were least effective in bringing down the leaf spot, gummy stem blight and wilt diseases. The plants raised from the seeds of fungal inoculation, irrespective of treatment showed reduced growth compared to corresponding control plants that were raised from the seeds free of target fungi. Topsin enhanced the overall growth and yield of the plants in spite of *A. cucumerina*, *M. verrucaria*, *M. roridum*, *D. bryoniae*, *F. oxysporum*, *F. solani*, *F. equiseti* and *F. verticilloides* infection. However, Topsin was found to be best for leaf spot causing fungi. In case of *D. bryoniae* infected plants Dithane M-45 treatment proved its efficacy in safe-guarding the plants and as the result there was increase in yield by four fold more over control. Next to Dithane M-45, Topsin showed its superiority in the enhancement of yield of the corp. Bavistin treatment improved the growth and yield of the crop against the infection of *Fusarium* species. In all the cases, Vitavax and Bayleton remained least effective against the fungal diseases and in the enhancement of yield parameter of the crop (Table 3). Pathogenic fungi invariably depend up on the host tissues for their nourishment. Their aggressiveness and establishment in the host tissue varies depending upon the ecological

conditions and other competing biological factors. In present study, the loss in emergence was quite severe due to fungi. This may be due to their heavy colonization on the growing tips of the seedlings, which might have facilitated the easy invasion of secondary pathogenic microbes. As the evidence there are certain reports indicated the loss of germination and seedling vigour of watermelon by *Fusarium oxysporum* [2]. Seedling diseases of watermelon due to infection of *Fusarium* species have been reported by McLaughlin and Martyn [3]. The present findings are also remain parallel with the earlier reports indicated the seedling mortality due to severity of *Fusarium* species. The pathogenic effect of *Myrothecium* species on the crop is also in support of the findings of Murthy *et al.* [4], who proved the adverse effect of *Myrothecium verrucaria* in teak saplings. *Myrothecium* species generally causes round dark brown leaf spots in cucurbits, which on later stages coalesce to form blighted area on the leaves [5]. Occurrence of leaf spot due to *Alternaria cucumerina* and Fusarium wilt by *Fusarium* species are reported by Wie *et al.* [6] and Sagir [7], respectively. The severity of the fungal diseases varies depending up on the prevailed whether conditions. Infected plants treated with Topsin was found to recover from the diseases. This may be due to the direct contact of the fungicides with the fungal propogules that developed on the symptom shown foliar regions. Pushpa *et al.* [8] explained the benefits of Bavistin to manage the seed-borne infection of *Fusarium*, *Colletotrichum* and *Alternaria* species in pumpkin, cucumber, muskmelon and watermelon. The wound through the spots, blights on older leaves of the plants might have facilitated the increased number of penetrating hyphae and higher conidial concentration during high humid condition. Svedelius and Unestam [9] have explained similar concept of development of disease propogules of *Didymella bryoniae* in cucumber leaves. The spray of plants with fungicides at the initiation of disease symptoms might have provided there direct contact with the fungal propogules and hence resulted in the poor establishment of the diseases. The effective fungicides might have protected the plants by triggering the plants to produce more phenolic compounds. The phenolic compounds produced by the plants are also known to inhibit the activity of cell wall degrading enzymes produced by pathogenic fungi [10]. As the consequences the developing parts might have recovered from the diseases, which ultimately enhanced the yield of the crop. Otherwise the chemical fungicides when they come in contact with the host tissue, the host cells might have triggered to produce more quantity of defense enzymes which includes peroxidases, chitinases, β-1,3 gluconases involved in the synthesis of phytoalexins [11, 12]. Hence, the effective fungicides might have played a role as inducers in the development of resistance of host plants against the infection of pathogenic fungi.

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