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Evaluation of Different Plant Protectants Against Seed Mycoflora of Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai]

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Abstract: In the present study, chemicals and antagonists were used for their efficacy in the management of seed-borne fungal pathogens of watermelon. The fungal species belonging to different genera were isolated from the seeds of watermelon and were used to assess their vulnerability to an array of chemicals and bio-agents. Among the fungal pathogens, *Fusarium* species was effectively suppressed due to Bavistin. Topsin also gave the promising results against all the fungal pathogens. Whereas, Dithane M-45 effectively controlled *Didymella bryoniae*. Seed treatment with antagonists like *Trichoderma harzianum* and *T. viride* improved the seed germination, seedling vigour and reduced the incidence of seed-borne fungal pathogens compared to control. Bavistin and Topsin among chemicals, *T. harzianum* among bio-agents increased the seed germination and vigour index significantly. *Trichoderma harzianum* showed its efficacy against all *Fusarium* species and even stood more effective than Captan and Blitox. However, *Pseudomonas fluorescens* also showed promising effect against *Didymella bryoniae* over fungicides.

Key words: Watermelon, fungicides, bio-agents, seed mycoflora, seed germination

Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], is an annual creeping, commercial crop grown throughout the world for its sugary, fleshy edible fruits. Though many commercial varieties have been released, farmers are facing severe loss of the crop due to microbial diseases in the field. Among microbes, fungi play an important role in causing leaf spots, blight, gummy stem blight, wilt and fruit rot. To over come these problems, seed treatment with chemical fungicides is only the solution. Routine and indiscriminate usage of fungicides are hazardous and hence, an alternate ecofriendly method to control these seed borne fungal pathogens is the use of bioagents. Based on the phytostimulatory and antifungal properties some of the microorganisms can be utilized in agriculture and horticultural practices. Though several reports indicated the use of bio-agents in many crops against the fungal diseases, information on biocontrol of seed-borne fungal pathogens in watermelon is not satisfactory. Hence, in the present investigations, seeds of watermelon were treated separately with three different bioagents such as *Trichoderma harzianum, T. viride and Pseudomonas fluorescens* and common fungicides such as Topsin, Bavistin, Dithane M-45, Captan and Blitox to assess their comparative efficacy in the inhibition of seed-borne fungi and growth promotion of the seedlings.

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Materials and Methods

Seed sample of Sugar Baby a popular variety of watermelon was collected from a seed company at Bangalore and was stored in polyethylene bag at 28±2° C. The seeds were then surface sterilized with NaOCl solution of 2% available chlorine for 5 minutes and washed 3-4 times with sterilized distilled water. To know the incidence of mycoflora, germination and seedling vigour, respectively, the surface sterilized seeds were then air dried and subjected to Standard Blotter Method, Paper Towel Method and Pot experiments in the seed testing laboratory at Mysore, University of Mysore, India.

Fungi such as *Alternaria cucumerina* (Ellis and Everhart) Elliot, *Myrothecium verrucaria* (Albertini and Schewein) Detmar ex Fr.., *Myrothecium roridum* Tode ex Fr., *Didymella bryoniae* (Awersw.) Rehm, *Fusarium oxysporum* Schlecht. Emend. Snyder and Hansen, *Fusarium solani* (Mart.) Apple and Wollenw. emend. Snyder and 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 inoculation.

Chemical fungicides viz., Topsin-M 70%WP [(Dimethyl 4,4,'-o-phenylenebis (3-thioallophanate)], Dithane M-45 75%WP (Manganese ethylene bis dithio carbamate plus zinc), Bavistin 50%WP (Methyl-H-benzimidazole-2ylcarbamate), Captan 50%WP [N-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide] and Blitox 50%WP (Copperoxychloride) were obtained from authorized agrochemical shops at Mysore in Karnataka. Seeds were dressed separately with these chemicals at the rate of 0.2% concentration. In all the cases untreated seeds served as control.

Fungal bio-agents *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers. ex Fr. and a bacterial agent *Pseudomonas fluorescens* were isolated from the rhizosphere of the maize plants and were cultured on Potato Dextrose Agar and Kings B broth. In case of fungi, 10 day-old cultures and in case of bacterium 2 day-old cultures were used to collect the respective inoculum. In case of fungi, cultures were agitated with sterilized distilled water using a soft camel hairbrush and the spore suspension was collected and centrifuged at 10,000 rpm for about 10 min. The sediment obtained was collected, air-dried and mixed with the talcum powder. The fungal spore load was adjusted to 78×10^6 spores/g seed and in case of bacterium the inoculum load was adjusted to 1×10^7cfu g^{-1} seed, using a spectrophotometer. The talcum formulation of each bioagents was used for the seed dusting at the rate of 15 g kg^{-1} seed. To know the effect of these chemicals and bioagents on the seed mycoflora, 400 seeds of each treatment were subjected to standard blotter method in which the seeds were plated, incubated according to the standard procedures of ISTA (Anonymous, 1996). On 8th day of incubation the seeds were evaluated for the incidence of mycoflora. For the purpose of assessing the effect of these treatments, 400 seeds of similar treatments were plated equidistantly on wet blotter sheets of size 9×14''. After plating the seeds, the blotter sheets were rolled into towels and incubated at 22±2°C under alternate cycles of 12/12 h light and darkness. After 14 days of incubation, seed germination percentage, root-shoot lengths of the seedlings were assessed and the vigour index was calculated (Abdul Baki and Anderson, 1973). 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

Data given in Table 1 indicated the varied response of fungi with respect to fungicides and bio-agents. Among the fungicides Topsin was found to be highly effective more than three fold in

Table 1: Effect of some fungicides and bioagents on seed mycoflora of watermelon var. sugar baby

Treatment	Percent incidence of mycoflora *Acremonium cucurbgacearume* ± SE	*Alternaria cucumerina* ± SE	*Didymella bryoniae* ± SE	*Fusarium oxysporum* ± SE	*Fusarium solani* ± SE	*Fusarium equiseti* ± SE	*Fusarium verticilloides* ± SE	*Myrothecium verrucaria* ± SE
Fungicides								
Topsin	3 ± 0.4^{f}	5 ± 0.4^{g}	18 ± 1.2^{de}	12 ± 1.5^{c}	14 ± 1.4^{cd}	11 ± 1.0^{fg}	8 ± 0.6^{de}	8 ± 0.3^{g}
Bavistin	5 ± 0.5^{ef}	9 ± 0.6^{de}	15 ± 1.3^{ef}	10 ± 1.7^{c}	12 ± 1.6^{d}	8 ± 0.6^{g}	6 ± 0.6^{e}	13 ± 0.6^{ef}
Dithane M-45	7 ± 0.6^{e}	7 ± 0.6^{fg}	13 ± 0.4^{f}	14 ± 0.9^{c}	17 ± 2.1^{bc}	12 ± 0.8^{ef}	9 ± 0.8^{d}	10 ± 1.1^{fg}
Captan	10 ± 0.9^{d}	12 ± 0.9^{d}	19 ± 1.4^{de}	18 ± 1.9^{b}	19 ± 0.4^{bc}	16 ± 1.1^{cd}	11 ± 0.6^{d}	12 ± 0.9^{f}
Blitox	8 ± 0.9^{de}	10 ± 0.8^{de}	22 ± 0.6^{cd}	20 ± 2.0^{b}	23 ± 2.2^{b}	$15\pm1.2^{c-e}$	14 ± 1.1^{c}	15 ± 0.8^{de}
Bio-agents								
Trichoderma harzianum	17 ± 1.0^{bc}	18 ± 1.4^{b}	24 ± 1.3^{bc}	14 ± 1.3^{c}	18 ± 2.1^{bc}	13 ± 0.3^{de}	10 ± 0.8^{d}	22 ± 0.9^{b}
Trichoderma viride	15 ± 0.5^{c}	16 ± 0.9^{bc}	27 ± 0.8^{b}	19 ± 1.4^{b}	21 ± 1.5^{b}	17 ± 0.9^{c}	8 ± 0.5^{de}	19 ± 1.1^{c}
Pseudomonas fluorescens	18 ± 1.1^{b}	15 ± 1.1^{c}	13 ± 1.1^{f}	24 ± 1.4^{b}	18 ± 1.4^{bc}	23 ± 1.3^{b}	18 ± 1.2^{b}	17 ± 0.4^{cd}
Control	30 ± 1.7^{a}	32 ± 1.3^{a}	63 ± 2.4^{a}	52 ± 1.3^{a}	58 ± 1.3^{a}	42 ± 2.1^{a}	26 ± 1.7^{a}	43 ± 1.6^{a}

Chemical fungicides were used at 0.2% concentration as dust formulations. Bio-agent formulation was used at 1.5% concentration. Data were recorded on the average of 200 seeds of four replicates for each treatment. SE = Standard Error of the Mean. According to Duncan's Multiple Range Test (DMRT) the values followed by different superscripts in the same column were significantly different at p = 0.05

Table 2: Effect of some fungicides and bioagents on seed germination and seedling vigour of watermelon var. sugar baby

Treatments	Seed germination (%) ± SE	MRL±SE (cm)	MSL±SE (cm)	VI±SE
Fungicides				
Topsin	90 ± 0.6^{a}	12.7 ± 0.06^{a}	15.6 ± 0.04^{a}	2549 ± 26^{ab}
Bavistin	93 ± 1.0^{a}	12.8 ± 0.08^{a}	15.8 ± 0.04^{a}	2641 ± 40^{a}
Dithane M-45	83 ± 0.9^{cd}	11.9 ± 0.09^{cd}	15.1 ± 0.1^{b}	2245 ± 41^{c}
Captan	87 ± 0.9^{b}	12.6 ± 0.06^{a}	15.5 ± 0.09^{a}	2458 ± 33^{b}
Blitox	81 ± 1.6^{d}	12.1 ± 0.1^{bc}	15.2 ± 0.1^{b}	2212 ± 63^{c}
Bio-agents				
Trichoderma harzianum	86 ± 1.1^{bc}	12.7 ± 0.09^{a}	15.8 ± 0.07^{a}	2446 ± 45^{b}
T. viride	82 ± 1.0^{d}	12.3 ± 0.1^{b}	15.0 ± 0.1^{b}	2223 ± 50^{c}
Pseudomonas fluorescens	75 ± 0.7^{e}	11.5 ± 0.08^{e}	13.9 ± 0.08^{c}	1913 ± 29^{e}
Control	78 ± 1.0^{e}	11.8 ± 0.1^{de}	14.4 ± 0.1^{c}	2048 ± 44^{d}

Chemical fungicides used at 0.2% concentration as dust formulations. Bio-agent formulation was used at 1.5% concentration. Data were recorded on 14 day of sowing, based on the average of 200 seeds of four replicates for each treatment. MRL = Mean Root Length, MSL = Mean Shoot Length, VI = Vigour Index and SE=Standard Error of the Mean. According to Duncan's Multiple Range Test (DMRT) the values followed by different superscripts in the same column were significantly different at p = 0.05

reducing the incidence of fungi over control. However, Bavistin was more effective against *Fusarium* species while Dithane M-45 reduced the incidence of *Didymella bryoniae* to a greater extent. Compared to all the fungicides, Blitox was found to be the least effective. Bio-agents also played an equivalent role in inhibiting a majority of fungi. *Trichoderma harzianum* and *T. viride* has proved its efficacy in reducing the incidence of many of fungi. However, *T. harzianum* was found to be more efficient against the *Fusarium* species. Whereas, *Pseudomonas fluorescens* showed promising effect over other bio-agents with respect to *D. bryoniae*, *Alternaria cucumerina* and *Myrothecium verrucaria*.

Topsin and Bavistin treated seeds also showed enhanced seed germination than any other treatment. Compared to control germination was increased by 12 and 13%, respectively. Blitox again remained in the last track with respect to seed germination and seedling growth. Seed treatment with biological agents like *T. harzianum* and *T. viride* also showed their efficacy by improving germination and root-shoot length of the seedlings, in case of paper towel method, which resulted in enhanced vigour index. *Trichoderma* sp. reached up to the level of chemical treatment and even stood superior over Captan and Blitox, while *P. fluorescens* was lagged behind with respect to germination and vigour index of the seedlings (Table 2). Hence, it can be concluded that the Topsin and *T. harzianum* treatment will be an efficient tool for the betterment of the plants in the field.

Microorganisms, as biological agents typically have a relatively narrow spectrum of activity compared with synthetic pesticides and often exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for the suppression of plant pathogens (Backman *et al*., 1997). In the seeds treated with Topsin, active molecules might have entered the seed tissues and interacted in a short time and paralysed the fungal mycelium. Whereas, other chemicals might not have penetrated sufficiently into seed tissues and hence, they might have failed to reach the target loci in the seed tissue. Chemical ingredient present in Topsin may be inhibitory to germination of fungal propogules. This is in opinion with the observations of Gupta and Shyam (1995) who have reported the antisporulant activity of some fungicides against the downy mildew of cucumbers. Enhanced germination due to promising chemicals may be confined to the suppression of pathogenic fungal species. Present findings are in support of the findings of Pushpa *et al*. (1999) and Keinath (2000) who have reported the beneficial effects of some fungicides against some of the seed-borne fungi causes severe epidemics. These chemicals perhaps are involved in the triggering up of the synthesis of defense enzymes like peroxidases, catalases, polyphenol oxydases, gluconases, chitinases, protease, lipases etc. and hence, safeguarded the host from the pathogenic fungal infection (M'pigo *et al.,* 1997). There also may be chances of degradation of phytotoxic compounds and hence, might have resulted in the normal growth of the plant. Similarly bio-agents might have triggered the host cells for the increased synthesis of phytohormones, which may be appreciably involved in the enhanced growth of the plants. These putative bio-agents might have also directly produced gluconases, chitinases etc., which might have played a role in the degradation of fungal propogules present in the seed tissues. The reduced germination and growth with respect to *P. fluorescens* treatment might be due to the high dose of the inoculum. Due to nutritional competence this treatment might have remained less promising over other treatment.

Uses of antagonistic microbes to control the fungal pathogens have been reported by various workers (Omar and Abd Alla, 1998; Sharma *et al*., 1998). The antagonism may be in the form of competition and hyperparasitism of cell lysis of susceptible ones. Indirect approach may be in the form of antifungal metabolites (Hendelsman and Eric, 1996). Similar results were reported by Abha and Tipathi (1999) in chickpea due to *T. viride* against *Botrytis*-gray mould. Secondary metabolites released from *Pseudomonas fluorescens* form complexes with iron, thus making non-availability of iron to other microbes. Siderophores are extracellular iron transporting agents known to produce by *Pseudomonas fluorescens,* form complexes with iron, thus making non-availability of iron to other microbes (Osburn, 1996).

Usually the mycoparasites produce an array of cell wall lytic enzymes, such as gluconases, chitinases, protease, lipases (Chet, 1987) or antibiotic metabolites such as gliotoxin and glioviridin (Di Pietro *et al*., 1993). The chitinolytic and glucanolytic enzymes or the combination of lytic enzymes and toxins of the mycoparasites usually act synergistically rather than alone (Chiu and Tzeam, 1995). *Trichoderma* species are capable of hyperparasiting on pathogenic fungi and found to be effective biocontrol agent for protecting a number of crop plants (Barnett and Binder, 1973). Bioagents posses remarkable capacity of multiplication, thus, when applied they multiply in exponential ratio and even can overcome stress condition by forming endospores or thick walled spores. Hence, bioagents may be considered as the solution for safer environmental issues, which needs proper attention while utilizing them for seed treatment. Over all evaluation of the present findings suggests the effective usage of Bavistin, Topsin and *T. harzianum* for the seed treatment of watermelon to overcome the seed-borne fungal infection.

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