African Journal of Biotechnology Vol. (38), pp. 6397-6400, 20 September 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.261
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Chemical control of blossom blight disease of sarpagandha caused by *Colletotrichum capsici*

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Accepted 5 August, 2010

Sarpagandha (*Rauvolfia serpentina* Benth) is grown in different parts of India and its adjoining countries for its root which is the chief source of several important alkaloids like ajmalicine, ajmaline, isoajmaline, rauvolfinine, reserpine and serpentine. Blossom blight caused by *Colletotrichum capsici* is one of the most serious diseases of sarpagandha in the North Indian plains. Eight fungicides, namely Benomyl, Carbendazim, Chlorothalonil, Mancozeb, Metalaxyl-mancozeb, Propiconazole, Thiram and Thiophanate-methyl were evaluated against the spore germination and mycelial growth of *Colletotrichum capsici* under *in vitro* condition. The results indicated that Mancozeb, Metalaxyl-mancozeb, Propiconazole and Thiram inhibited percent spore germination at the dose of 2 - 10 µg/ml, while Benomyl, Carbendazim and Thiophanate-Methyl were ineffective even at 250 µg/ml concentration. Propiconazole, Carbendazim, Benomyl, Mancozeb and Metalaxyl-mancozeb were highly effective inhibiting 50% mycelial growth at 2.8, 4.6, 6.0, 9.3 and 11.2 µg/ml, respectively, while Thiophanate-methyl, Chlorothalonil and Thiram were relatively less effective showing 50% mycelial growth inhibition at 25.2, 27.2 and 31.3 µg/ml, respectively. The effective fungicides were further employed for the protection of sarpagandha from blossom blight disease in the field during the year 2006 and 2007. The results of the two years (2006 and 2007) of field experiments indicated that disease incidence was significantly reduced in all the treated plots over unsprayed control. The spray of Mancozeb and Carbendazim produced highest protection that is >80% in both the years against blossom blight disease and their treatments also produced healthy and highest seed yield per plant.

Key words: Blossom blight, *Colletotrichum capsici*, fungicides, *Rauvolfia serpentina*, sarpagandha.

INTRODUCTION

Sarpagandha (*Rauvolfia serpentina* Benth .Ex.Kurz family Apocynaceae) is growing in different parts of India and its adjoining countries. Its root contains many important alkaloids, like ajmalicine, ajmaline, isoajmaline, rauvolfinine, reserpine, serpentine, rescinnamine, tetra-phylicine, yohimbine and 3 epi α-yohimbine (Snimolia et al., 1984). Sarpagandha has been used in several Ayurvedic formulations from ancient times. Its extract served as an antidote for the bites of poisonous reptiles and insects, and also used as hypotonic and sedative in various disorders, in anxiety states, excitement, maniacal behavior associated with psychoses, insanity, insomnia and epilepsy. Blossom blight caused by *Colletotricum capsici* is one of the most important diseases of sarpagandha in North Indian plains and caused drastic reduction in the production of healthy seeds (Shukla et al., 2006). The disease incidence adversely affects the sarpagandha plantation because of severe infection on inflorescence leading to premature death of the infected flowers. The blossom blight also resulted in decapitation and prevented seed setting.

There are many reports on the chemical control of the diseases caused by the pathogen, *C. capsici* on other hosts (Mishra, 1988; Eswaramurthy et al., 1988; Ekbote, 2005) but the work on chemical control of blossom blight disease of sarpagandha has not been carried out so far. Thus, attempts have been made to evaluate the efficacy of different fungicides against the pathogen, *C. capsici in-vitro* and apply effective fungicides for two consecutive

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Table 1. Minimum inhibition concentration (MIC) and EC-50 values of different fungicides against spore germination and mycelial growth of *Colletotrichum capsici*.

Fungicides	Spore germination		Mycelial growth	
	MIC (µg/ml)	EC-50 (µg/ml)	MIC (µg/ml)	EC-50 (µg/ml)
Benomyl	> 250**	-	> 5 < 100	6.0
Carbendazim	> 250	-	> 5 < 100	4.6
Chlorothalonil	> 5 <50	27.5	> 5 < 250	27.2
Mancozeb	> 3 <10	3.5	> 5 < 100	9.3
Metalaxyl-mancozeb	> 3 <10	4.2	> 5 < 100	11.2
Propiconazole	> 2 < 10	2.8	> 2 < 10	2.8
Thiram	> 3 < 10	3.0	> 5 < 250	31.3
Thiophanate-methyl	> 250	-	> 5 < 250	25.2

*All the *in vitro* effects were calculated based on data from five Petri dishes.
**Supported spore germination but inhibited mycelial growth even at very low dose.

years for the protection of sarpagandha from blossom blight disease in the field and results are reported.

MATERIALS AND METHODS

Fungicides

Benomyl (Benlate 50% WP, E.I DuPont India Ltd, New Delhi-17, India), Carbendazim (Bavistin 50% WP, BASF India Ltd, Thane-Belapur Road, Thane, Mumbai, India), Chlorothalonil (Kavach 75% WP, Syngenta India Ltd., Mumbai-20, India), Mancozeb (Dithane M-45, 75% WP, Indofil Industries Ltd., Bari Brahamana, Jammu, India), Metalaxyl-mancozeb (Ridomil MZ 72% WP, Syngenta India Ltd., Mumbai-20, India), Propiconazole (Tilt 25% EC, Syngenta India Ltd., Mumbai-20, India), Thiram (Thirox 75% WS, Crop Chemicals Ltd., Ernakulam, Cochin-031,India) and Thiophanate–methyl (Topsin–M 70% WP, Motilal Pesticides Pvt Ltd., Masani, Mathura-003, India) were used to test their activity *in-vitro* and effective ones were applied in the field to control blossom blight disease of sarpagandha.

Evaluation against spore germination and mycelial growth of *C. capsici*

For testing spore germination inhibition, spore of *C. capsici* (1x106 spores ml-1) were treated by different concentrations (5 to 250 ug/ml) of fungicide solution. The spores treated with sterile distilled water served as control. One ml treated as well as non-treated (control) spore suspension was spread over thin poured 2% water agar medium in 5 replicated Petri dishes (90 mm) and incubated at 23 ± 2°C. All the tests and treatment were repeated twice. Percentage of spore germination was recorded after 16 h and percentage of spore germination inhibition over control was determined in each of the treatments.

Based on active ingredients, the required quantity of different fungicides was incorporated individually into melted potato-dextrose-agar (PDA) to obtain 5, 10, 50, 100 and 250 µg/ml concentration. The medium without fungicide served as control. Aliquot of 20 ml of each fungicide added medium was poured into 90 mm Petri dishes with 5 replicates. They were later inoculated by 6 mm diameter. mycelial disc from 7 day- old- culture of *C. capsici* and incubated at 23 ± 2°C. Mycelial growth inhibition was determined after 6 days and EC-50 (effective concentration for inhibiting 50% radial growth) values were determined by the method of Bliss (1934) and Vincent (1947).

Effective chemical control of blossom blight disease of sarpagandha in the field

Five fungicides showing effective performance under *in-vitro* evaluation were used in different doses. The experiment was carried out for the two consecutive years (2006 and 2007) in complete randomized block design with plot size 3 x 2 m^2 at CIMAP experimental field, Lucknow. The soil type of the experimental field was sandy loam; standard package of practices for *R. serpentina* recommended for North Indian plains were followed during the course of study (Khanuja et al., 2005). Sarpagandha plantations were carried out in the field at a distance of 45 x 60 cm in the month of February 2005 - 2006. All the treatments were performed in triplicate. First spray was given just after appearance of initial symptoms of the disease on the flowers in second week of July. Thereafter; 3 more applications were done at the interval of 15 days till 2nd week of September. Tween-20 (Hi-Media Laboratories Pvt. Ltd Mumbai India) at 0.25 ml/l was added to the fungicide solution as a spreader/sticker during rainy season. The effectiveness of fungicides was evaluated 15 days after the fourth application by rating infection on the inflorescence of the infected plants in each of the plots on a 0 - 4 scale (Shukla et al., 2006). The percent disease index (PDI) and percent disease control (PDC) was calculated by the formulae evolved by McKinney (1923) and Chester (1950), respectively. The data were analyzed statistically and critical difference (CD) was calculated at 5%.

RESULTS

Effect of fungicides on the spore germination and mycelial growth of *C. capsici*

Results shown in Table 1 indicated that minimum inhibition concentrations (MICs) of the fungicides against spore germination were invariably less than those against the radial mycelial growth. Propiconazole was most effective as it inhibited 91.1% spore germination of *C. capsici,*

Table 2. Chemical control of sarpagandha blossom blight (%) and seed yield/plant in the field.

Fungicides	Doses (%)	2006			2007		
		Disease incidence (%)	Disease control (%)	Average seed yield* g/plant	Disease incidence (%)	Disease control (%)	Average seed yield* g/plant
Control	_	52.84	_	1.08	54.97	-	1.37
Benomyl	0.15	13.63	74.21	5..56	15.02	72.64	8.87
Carbendazim	0.15	8.00	84.86	8.89	9.02	83.59	10.15
Mancozeb	0.30	6.43	87.83	11.67	8.78	84.03	13.61
Metalaxil-mancozeb	0.15	11.75	77.67	6.48	13.71	75.06	8.45
Propiconazoe	0.15	21.53	59.25	4.14	_	_	_
S Em	_	1.48	_	0.51	1.23	_	0.54
CD. at 5%	_	4.67	_	1.60	3.87	_	1.69

*Average seed yield was calculated from 5 plants with 3 replicates.

followed by Thiram (64.1%), Mancozeb (60.9%), and Metalaxly-mancozeb (58.1%) at 5 µg/ml, while Benomyl, Carbendazim and Thiophanate-methyl were ineffective as their treatment failed to inhibit spore germination even at 250 µg/ml concentration. Propiconazole, Carbendazim, Benomyl, Mancozeb and Metalaxyl-mancozeb inhibited 50% mycelial growth at 2.8, 4.6, 6.0, 9.30 and 11.3 µg/ml, respectively, while Ec-50 values for Chlorothalonil, Thiram and Thiophanate-methyl were 27.2, 31.3 and 25.2 µg/ml, respectively.

Effective chemical control of blossom blight disease of sarpagandha in the field

During the year 2006, four sprayings with each of five selected fungicides (Table 2) over the flowers and foliage of the sarpagandha plants in the field caused significant reduction disease incidence over untreated control. Mancozeb and Carbendazim were most effective as their treatment produced 87.83 and 84.86% protection, respectively over untreated control. Metalaxyl-mancozeb and Benomyl were the next effective fungicides producing 77.67 and 74.21% protection but the effect was not significantly different in between these two fungicides. Propiconozole was least effective producing 59.25% disease control. For this reason it was not used in the year 2007.

Results of the year 2007 are shown in Table 2.where four fungicides, namely Benomyl, Carbendazim, Mancozeb and Metalaxyl-mancozeb were applied in the field. Mancozeb and Carbendazim produced almost similar results as in 2006. They exhibited 8.78 and 9.02% disease incidences and highest protection that is 84.03 and 83.59%, respectively over control (Figure 1). The disease incidences in control plots were recorded to be 52.84 and 54.97% in 2006 and 2007, respectively. The protection due to fungicide application was significant, although the efficacy of different fungicides differ from each other. Our experimental results (Table 2) also indicated that seed yield/plant was increased significantly over control due to fungicidal spray. The spray of Mancozeb and Carbendazim in the year 2006 and 2007 invariably produced maximum seed yield/plant that is 11.67-13.61 g and 8.89 - 10.15 g, respectively. In the unsprayed control plots, plants either produced very little amount of seeds/plant or seed setting was not at all present.

DISCUSSION

The *in vitro* effect of fungicides did not match with their performances *in vivo* against blossom blight disease of sarpagandha. For instance, Carbendazim did not have high effect against mycelial growth and spore germination of the pathogen at lower concentrations but its field performance was effective and significant. It has inhibited conidial germination of *C. capsici* at higher concentrations thereby produced effective control of the disease in the field. Mancozeb was highly effective against conidial germination at lower concentrations but not against the mycelial growth. It gave promising protection from *C. capsici* infections in the field (Figure 1). The control of anthracnose and fruit rot disease of chili caused by *C. capsici* was achieved by spraying with Carbendazim and Mancozeb (Das and Mohanty, 1988; Biswas, 1992; Ebenezar and Alice, 1996). Sometimes, fungicide highly effective *in vitro* was not effective *in vivo*, as we observed with Propiconazole. It was highly effective against spore germination and mycelial growth of *C. capsici* but was ineffective in the field when sprayed over the flowers and foliage. Such finding was also reported by Margina and Zheljazkov (1994). It is attributed that this may be due to non-absorption/decomposition by the plant.

Figure 1. Effective control of blossom blight disease of sarpagandha with spraying by Mancozeb under field condition.

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