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Seven fungicides were evaluated against *Phytophthora parasitica* f.sp. *nicotianae,* the causal agent of leaf blight disease in tobacco nurseries, under *in vitro* and *in vivo*. Among the fungicides evaluated, fenamidone + mancozeb 60% WG was most effective. It inhibited the pathogen growth up to 100% at 100 ppm. It was followed by metalaxyl + mancozeb and pyraclostrobin + metiram at 1000 ppm concentrations. *In vivo* nursery evaluation of fungicides, indicated that two sprays one at 21 days followed by another at 31 days after sowing with fenamidone + mancozeb 60% WG @ 0.3 per cent was effective in checking the disease incidence and increased the transplantable seedlings of FCV tobacco with higher monetary returns.

Key words: Fungicides, leaf blight, *Phytophthora parasitica* f. sp. *nicotianae,* virginia tobacco

INTRODUCTION

Tobacco is one of the important commercial crops of India and contributes about Rs. 6,000 crores as foreign exchange and Rs. 21,000 crores revenue to the national exchequer annually. Diseases caused by fungal pathogens are major constraints in successful production of this exportable commodity. *Phytophthora parasitica* f. sp*. nicotianae* (Breda de Haan) Tucker*, c*ausing Leaf blight disease in tobacco nurseries is a major problem in the production of quality tobacco seedling . Heavy mortality of the seedlings in all types of tobaccos including Virginia tobacco nurseries due to this pathogen is a cause of concern.

Metalaxyl + mancozeb have been recommended and are in use since three decades for the management of this disease in tobacco nurseries. To avoid resistance development and to find out alternative fungicides, it is essential to assess new fungicides, for their bio-efficacy against leaf blight disease. Efforts are being constantly made to evaluate new class of fungicides for developing efficient IPM strategies. The present studies on evaluation of a new fungicides against the pathogen, *Phytophthora parasitica f. sp. nicotianae,* the incitant of leaf blight disease in FCV tobacco nursery are carried out with a view to select the most effective fungicide for the management of the disease.

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MATERIALS AND METHODS

In vitro studies

Poisoned food technique was followed to study the comparative efficacy of 7 fungicides at 100, 250, 500 and 1000 ppm of formulation against the virulent isolate of *Phytophthora parasitica* f. sp. *nicotianae* in five replications. Required concentration of each fungicide from the commercial formulations was prepared with sterilized distilled water and added to autoclaved potato dextrose agar (PDA) medium to obtain desired dilutions. The medium without fungitoxicant served as control. The Petri dishes containing PDA medium were inoculated with 5 mm discs from two days - old actively growing culture of *P. parasitica f. sp. nicotianae* grown on PDA, the inoculated Petri dishes were incubated at 28 ± 2^0 C temperature and growth of the mycelial colony was measured 9 days after inoculation. Extent of inhibition of mycelial growth by each fungicide was calculated by estimating the per cent reduction in mean mycelial diameter

over that of the control. The data were subjected to statistical analysis.

Nursery experiment

The nursery experiment was conducted for two seasons 2014 and 2015 at ICAR- Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh to test the bio-xs nursery season (September to November).

Seven fungicides *viz*., azoxystrobin 23% SC, trifloxystrobin + tebuconazole 75% WG, pyraclostrobin + metiram 60% WG, kresoxim methyl 44.3% SC, fenamidone + mancozeb 60% WG, metalaxyl + mancozeb 72% WP and copper oxychloride 50% WP were applied to the nursery as spray @ 100 ml/m^2 at 21 and 31 DAS along with recommended check for evaluation under nursery conditions. The experiment was laid out in a randomized block design (RBD) with three replications with a plot size of 1 m^2. The popular cultivar *Siri* was used in the experiment. Regular disease observations on leaf blight and phytotoxicity were recorded, while healthy transplant count was recorded at each pulling of the seedlings. Germination count was taken at 15 days after sowing (DAS) at random in ten squares each with a dimension of 100 sq.cm. from which mean was calculated.

RESULTS AND DISCUSSION

***In vitro* studies**

All the seven fungicides evaluated against *Phytophthora parasitica* f. sp. *nicotianae* were found inhibitory to the fungus with varied degree of inhibition and the data are presented in Table 1. The results indicated that out of seven fungicides evaluated against the test pathogen, fenamidone + mancozeb 60% WG was the most effective as it checked cent per cent growth of fungus even at 100 ppm which was followed by metalaxyl + mancozeb 72% WP and pyraclostrobin + metiram 60% WG at 1000 ppm concentrations, respectively. Whereas, copper oxychloride 50% WP, trifloxystrobin + tebuconazole 75% WG, kresoxim methyl 44.3% SC and azoxystrobin 23% SC were found to be less effective even at 1000 ppm concentrations. With the rise in concentration from 100 to 1000 ppm, effectiveness of the fungicides in respect to checking the mycelial growth also increased in all the cases. Earlier reports also suggested fenamidone + mancozeb and metalaxyl + mancozeb to have cent per cent inhibitory effect on mycelial growth of *Phytophthora capsici* & *ramorum* under laboratory conditions.

Nursery experiment

Pooled means of treatments for two seasons showed significantly lower disease index as compared to untreated check. Eight treatments including positive check and untreated check were evaluated against leaf blight disease and the data are presented in Table 2. Spraying with fenamidone + mancozeb 60% WG @ 0.3 per cent at 21 and 31 days after sowing of nursery recorded maximum per cent disease reduction of 89.75% followed by metalaxyl + mancozeb 72% WP @ 0.2 % (67.08%). Among the new fungicides, azoxystrobin @ 0.1% (54.34%), pyraclostrobin + metiram @ 0.2% (58.07%), kresoxim methyl @ 0.1% (49.06%) and trifloxystrobin + tebuconazole @ 0.1% (40.68%) did not perform well in checking the incidence of the disease. All the fungicides were superior to untreated check with more than 40 per cent reduction of disease over control. Maximum disease incidence (3.22) was noticed in untreated check.

The effectiveness of fenamidone + mancozeb was reported by Khadka *et al* (2016) in potato against late blight disease (*Phytophthora infestans*) from Western Nepal, Dangi (2016) from Michigan State University and Lal *et al* (2015) and Kumar *et al* (2012) in India.

There was no significant difference with respect to germination at 15 Days after sowing among the different treatments. The significant control of leaf blight disease has been reflected on the yield of healthy transplants. Higher numbers of transplantable seedlings were recorded in fenamidone + mancozeb (836/m^2) @ 0.3% spray followed by metalaxyl + mancozeb (772/m^2) @ 0.2%. All other fungicides showed higher number of healthy transplantable seedlings when compared to untreated check (291/m^2). The fungicide did not show any phytotoxicity even at 3 days after spraying.

The economic analysis (Table 3) revealed that the highest net returns of Rs. 96,372 per ha were obtained with an ICBR of 1:12.35 in fenamidone + mancozeb 60% WG spray @ 3g/l. The study clearly indicated the economic advantage of using fenamidone + mancozeb spray.

Table 1: *In vitro* evaluation of different fungicides against *Phytophthora parasitica* f. sp. *nicotianae*

Fungicides	% inhibition of mycelial growth over control/ concentrations (ppm)				Mean
	100	250	500	1000	
Copper oxychloride 50% WP	62.44 (52.19)	92.66 (74.26)	93.33 (75.00)	94.22 (77.50)	85.66 (69.74)
Metalaxyl + Mancozeb 72% WP	87.78 (69.53)	93.10 (74.75)	97.33 (83.97)	100 (90.00)	94.55 (79.55)
Trifloxystrobin + Tebuconazole 75% WG	28.00 (31.93)	30.88 (33.74)	44.89 (42.04)	65.33 (53.90)	42.27 (40.40)
Pyraclostrobin + Metiram 60% WG	86.44 (68.38)	89.77 (71.33)	93.33 (75.00)	100 (90.00)	92.38 (76.17)
Fenamidone + Mancozeb 60% WG	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Kresoxim Methyl 44.3% SC	27.55 (31.62)	27.55 (31.62)	27.78 (31.78)	27.78 (31.78)	27.66 (31.71)
Azoxystrobin 23% SC	26.22 (30.78)	26.44 (30.92)	26.44 (30.92)	28.44 (32.21)	26.89 (31.24)
Mean	59.77 (50.67)	65.77 (54.17)	69.01 (56.25)	73.68 (59.03)	67.06 (54.97)
	Fungicides (F)	Conc. (C)	F x C		
S. Em ±	0.50	0.78	0.99		
C. D (p=0.05)	1.38	1.04	2.76		

Figures in parentheses are angular transformations

Table 2: Efficacy of new fungicides in the management of leaf blight disease of FCV tobacco Nursery

Fungicides	Incidence of leaf blight diseased seedlings/m^2			% reduction of disease over control	Germination at 15 DAS*/m^2			Total healthy transplant/m^2		
	2014-15	2015-16	Pooled		2014-15	2015-16	Pooled	2014-15	2015-16	Pooled
Azoxystrobin 23% SC @ 0.1%	23.33 (4.80)	16.33 (4.03)	19.83 (1.47)	54.34	43.00	47.66	45.33	702	727	714
Trifloxystrobin + Tebuconazole 75% WG @ 0.1%	29.66 (5.35)	38.33 (6.15)	34.00 (1.91)	40.68	52.33	46.33	49.33	679	617	648
Pyraclostrobin + Metiram 60% WG @ 0.2%	11.33 (3.29)	24.00 (4.86)	17.66 (1.35)	58.07	47.33	51.66	49.50	676	670	673
Kresoxim methyl 44.3% SC @ 0.1%	25.00 (4.98)	25.67 (4.88)	25.33 (1.64)	49.06	53.33	48.33	50.83	669	678	674
Fenamidone + Mancozeb 60% WG @ 0.3%	0.00 (0.00)	6.67 (2.03)	3.33 (0.33)	89.75	51.00	44.33	47.66	850	822	836
Metalaxyl + Mancozeb 72% WP @ 0.2%	8.66 (2.93)	12.33 (3.44)	10.49 (1.06)	67.08	48.33	48.00	48.16	796	748	772
Copper oxychloride 50% WP @ 0.2%	13.00 (3.60)	25.67 (4.60)	19.33 (1.36)	57.76	42.66	48.00	45.33	599	507	553
Untreated check	70.66 (8.28)	128.67 (11.06)	99.66 (3.22)	-	54.33	43.33	48.83	275	308	291
Sem±	0.53	1.06	0.08	-	2.85	2.46	1.34	24.58	26.04	5.45
CD (p=0.05)	1.61	3.24	0.25	-	NS	NS	NS	74.57	78.99	15.77
S x T interaction SEm±	-	-	0.12	-	-	-	1.90	-	-	7.70
CD (p=0.05)	-	-	0.35	-	-	-	5.50	-	-	22.31

Figures in parentheses are SQRT transformation values

* DAS: Days after Sowing

Table 3: Economics of leaf blight disease control in FCV tobacco nurseries with different fungicides

Fungicides	Transplantable seedlings/ha	Net returns (Rs/ha)	ICBR
Azoxystrobin 23% SC @ 0.1%	40,56,234	53,196	1:7.59
Trifloxystrobin + Tebuconazole 75% WG @ 0.1%	36,81,288	26,741	1:3.57
Pyraclostrobin + Metiram 60% WG @ 0.2%	38,23,313	37,991	1:7.59
Kresoxim methyl 44.3% SC @ 0.1%	38,28,994	38,289	1:7.65
Fenamidone + Mancozeb 60% WG @ 0.3%	47,49,316	96,372	1:12.35
Metalaxyl + Mancozeb 72% WP @ 0.2%	43,85,732	76,761	1:23.26
Copper oxychloride 50% WP @ 0.2%	31,41,593	22,932	1:17.64

ICBR: Incremental cost benefit ratio

From the *in vitro* studies and the nursery experiments conducted for two seasons it may be inferred that two sprays one at 21 days followed by another at 31 days after sowing with fenamidone + mancozeb 60% WG @ 0.3 per cent were highly effective in terms of disease control, increasing transplantable seedlings of FCV tobacco as well as favourable economics, and hence can be recommended for management of the leaf blight disease in tobacco nurseries as an alternative to metalaxyl + mancozeb 72% WP @ 0.2%.

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