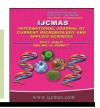


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Evaluation Fungicides against *Phytophthora nicotianae* Causing Black Shank Disease in FCV Tobacco Both Under *In vitro* and *In vivo*

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ABSTRACT

Keywords

Phytophthora nicotiane, Black shank, FCV tobacco, Fungicides, Management.

Article Info

Accepted: 26 June 2017 Available Online: 10 July 2017 Black shank, *Phytophthora parasitica* var. *nicotianae* is a major disease of tobacco results in considerable loss of the crop. An investigation was carried out to screen the different fungicides to inhibit the growth of black shank pathogen. Among eight fungicides tested at different concentrations, 0.1 per cent of Trifloxystrobin + Tebuconazole, Fenamidone + Mancozeb and Metalaxyl + Mancozeb were showed cent per cent inhibition and found effective over all the tested fungicides. Management study under field conditions was carried out during *kharif* 2016, revealed that the treatment with Fenamidone + Mancozeb and Cymoxanil + Mancozeb found effective in reducing the disease incidence of 19.05% and 23.02% with highest green leaf yield (10596.71 and 9920.63 kg/ha), cured leaf yield (1246.67 and 1167.13 kg/ha) and top grade equivalent (872.67 and 700.28 kg/ha) followed by Azoxystrobin and Metalaxyl Mz over untreated check. All tested fungicides were drastically reduced disease the incidence and showed a tendency to increase tobacco yield over the untreated control.

Introduction

Among the commercial crops grown in India, tobacco (*Nicotiana tabacum*) occupies an important place not only as a valuable foreign exchange earner but also as an employment as a generating crop. Tobacco like any other crop is susceptible to several diseases in which black shank is one of the serious problem of Flue Cured Virginia tobacco in Karnataka light soil. Black shank of tobacco, caused by the hemibiotrophic oomycete *Phytophthora parasitica* f. sp. *nicotianae* (Breda de Haan) Tucker is among the most destructive and widespread of all

tobacco-cultivated area (Prinsloo, 1994). The Pathogen damage the pith (the water transport system of the plant) of a plant resulted wilting, stunting, yellowing leaves and finally plant dies (Lucas, 1975). Black shank first appeared in the United States in 1915 and was first reported in North Carolina in Forsyth County in 1931. The fungus can survive in the soil for many years, even in the absence of tobacco (Shew and Lucas, 1991; Jones and Shew, 1995; Shamrao and Hundekar, 2009). The disease can be managed in many ways. For effective black shank control, growers use

a combination of crop rotation, cultivar resistance and fungicide applications (Melton 1998). Application of effective fungicides continues to be a viable component in developing integrated disease management programme. In this context the present investigation was carried out on management of black shank disease by using different fungicides under natural field condition.

Materials and Methods

In vitro evaluation of fungicides against P. nicotiane

The study was conducted at Plant Pathology department, University of Agricultural and Horticultural Scinces, Shivamogga during 2016. The efficacy of two systemic and six combi fungicides viz., Fosetyle Al (Allite), Azoxystrobin 23EC (Amistar), Zineb 68% + Hexaconazole 4% WP (Avtar), Cymaxonil 8% + Mancozeb 65% (Curzate), Famoxadone 16.6%+ Cymoxanil 22.1 % (Equation Pro), Fenamidone 10% + Mancozeb 50 WG (Sectin 60 WG), Trifloxystrobin 25% Tebuconazole 50% WG (Nativo) and Metalaxyl 8% + Mancozeb 64% WP (Rodomil gold) were tested at 0.1, 0.15 and 0.2 per cent concentration against *P. nicotiane* for radial growth inhibition on the potato dextrose agar media using poisoned food technique under in vitro condition.

The quantity of fungicides was calculated for 100 ml medium separately. The requisite quantity of fungicides was added to each flask at 45 C. the fungicides were thouroghlly mixed before solidification and poured into sterilized Petri plates. The mycelium of 5mm diamere of five days old culture was cut with the help of sterile corkborer. Each disc was transferred aseptically to the centre of each Petri plate, already poured with poisoned medium. The PDA plates without fungicides were also inoculated and maintained as

control. The plates were incubated for 6 days. The observations on colony growth was recorded until Petri plate in control treatment was fully covered with mycelia growth and calculated the per cent inhibition by using the formula given by Vincent (1947).

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment (fungicide)

Evaluation of fungicides against black shank of FCV tobacco under field condition during *kharif* 2016

The experiment was carried out during *kharif* 2016 at Zonal Agricultural Research Station, Navile, Shimoga, Karnataka. The soil type was loamy soil and had the black shank pathogen (*Phytophthora nicotiane*) population to an extent of 6 x 10⁶ cfu/g of soil. The experimental plot size was 5.4 x 4.0 m with seven treatments of three replication and laid out in Randomized Complete Block Design.

The variety, Sahaydri was used and plots was (50ml/plant) drenched with different fungicides viz., Fosetyl Al @ 0.1%, Zineb 68% + Hexaconazol 4% WP @ 0.2%, Trifloxystrobin 25% and Tebuconazole 50 %WG @ 0.1%, Cymoxanil 8% + Mancozeb 64% WP @ 0.2%, Famoxadone 16.6%+ Cymoxanil 22.1 % @ 0.1%, Azoxystrobin 23 EC @ 0.1%, Fenamidone 10% + Mancozeb 50 WG @ 0.2%, Metalaxyl 8% + Mancozeb 64% WP @ 0.2% (RC) and one check. These plots were watered by surface irrigation at regular intervals (6 to 8 days) throughout the season and stopped 15 days before harvesting. An observation on disease incidence was

scored using 0-5 scale, and yield parameters *viz.*, green leaf weight, cured leaf weight and total grade equivalent (TGE) were recorded.

Statistical Analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and randomized block design. Data from the percent inhibition, percent disease incidence and yield were analyzed by ANOVA. Percent data were transformed arcsine where necessary. Differences within the means were compared by using Fisher's LSD (Least Significant Difference) test (Steel *et al.*, 1997).

Results and Discussion

In vitro evaluation of fungicides against Phytopthora nicotiane

It was revealed from the results (Table 1) that all systemic and combi fungicides at different significantly concentrations inhibit the mycelial growth of *P. nicotiane*. The fungicides, Fenamidone + Mancozeb, Cymoxanil + Mancozeb, and Metalaxyl + Mancozeb at 0.1, 0.15 and 0.2 per cent concentrations showed cent percent inhibition and remaining were showed more than 50 per cent mycelial inhibition at all the tested concentration. However, the Fenamidone + Mancozeb, Cymoxanil + Mancozeb, and Metalaxyl Mancozeb all concentration caused 100 percent reduction of mycelail growth followed by Azoxystrobin (79.56%), Trifloxystrobin + Tebuconazole Famoxadone + Cymoxanil (79.11%),(78.67%) Zineb + Hexaconazole (73.33%), and Fosetyl Al (70.22%) at 0.2 percent concentration respectively. It was observed from the study that amongst tested fungicides combi fungicides found significantly superior

in showing cent percent inhibition of the mycelial growth of the *P.nicotiane*. Jahagirdar (1998) reported the fungicidal nature of Akomin, a plant tonic generally being recommended for plantation crops. The laboratory evaluation of Ridomil against P. parasitica var. nicotianae revealed significant reduction in growth and sporulation of fungus at 0.1, 0.2, 0.3 and 0.4 per cent concentration. Sastry (1982) reported Bordeaux mixture (1%), Blitox and Metalaxyl which were found effective in inhibiting the growth and sporangial formation of P. capsici and P. meadii. Similar reports in in vitro screening were reported by Ramachandran and Sarma (1990), Ramachandran et al., (1990). The present investigation results are also in line with the findings of Subramanyam (1993), Jahagirdar (1998) and Veena and Sarma (2000). Studies on metalaxyl as effective fungicide agent P. parasitica var. nicotianae causing black shank of tobacco was reported by Vasilakakis et al., (1979).

Evaluation of fungicides against black shank of FCV tobacco under field condition during *kharif* 2016

Eight different fungicides (two systemic and six combi) present in the local market were tested for their antifungal potential against P. nicotiane under natural field conditions in curative manner. The incidence of black shank as well as the green leaf yield cured leaf yield and total grade equivalent (TGE) varied with the application of different new molecules of fungicides in field condition. Soil application of fungicides during and after planting, reduced the disease incidence and increased the yield parameters. The results from table 2 reveal that, all the tested fungicides were significantly effective in reducing the disease incidence. Maximum reduction of the disease incidence of 19.05% and 23.02% were recorded application of Fenamidone + Mancozeb at 0.3%

Cymoxanil + Mancozeb at 0.2% as compared untreated control (76.19%) respectively. Further 0.1% of Azoxystrobin (25.40%) 0.1% of Metalaxyl + Mancozeb (30.95%) and Fosetyl Al (34.92%) found effective in reducing the disease incidence followed by Least percent disease reduction was recorded in Famoxadone + Cymoxanil at 0.1% and Zineb + Hexaconazole of 33.34 and 34.48% over control. Correspondingly, Fenamidone + Mancozeb and Cymoxanil + Mancozeb

applied plots yielded higher green leaf of 10596.71 and 9920.63 kg/ha, cured leaf of 1246.67 and 1167.13 kg/ha and top grade equivalent of 872.67 and 700.28 kg/ha respectively over untreated check (5291.01, 622.47 and 497.98 kg/ha). Therefore the combi fungicide Fenamidone + Mancozeb was found most effective in reducing the black shank disease in FCV tobacco and thereby increasing the leaf yield.

Table.1 Evaluation of fungicides against *Phytopthora parasitica* var. *nicotiane* under *in vitro*

	Pe	Mean			
Fungicides	Co				
	0.1	0.15	.15 0.2		
Fosetyl Al	54.22	64.00	70.22	62.81	
Tosciyi Ai	(47.45)	(53.16)	(56.98)		
Zineb 68% +	66.67	69.33	73.33	69.78	
Hexaconazole 4% WP	(54.76)	(56.40)	(58.94)	09.76	
Cymoxanil 8% +	100.00	100.00	100.00	100.00	
Mancozeb 64% WP	(90.05)	(90.05)	(90.05)		
Trifloxystrobin 25%	60.89	68.89	79.11	69.63	
+Tebuconazole 50 %WG	(51.32)	(56.13)	(62.95)	09.03	
Famoxadone 16.6%+	59.11	62.22	78.67	66.67	
Cymoxanil 22.1 %	(50.28)	(52.10)	(62.61)	00.07	
Azovystrobin 22 EC	54.67	60.00	79.56	79.56 (63.31) 64.74	
Azoxystrobin 23 EC	(47.70)	(50.79)	(63.31)		
Fenamidone 10% +	100.00	100.00	100.00	100.00	
Mancozeb 50 WG	(90.05)	(90.05)	(90.05)	100.00	
Metalaxyl 8% + Mancozeb	100.00	100.00	100.00	100.00	
64% WP	(90.05)	(90.05)	(90.05)	100.00	
	Fungicides	Concentration	I (F x C)		
	(F)	(C)	I (I A C)		
S.Em±	1.66	1.02	2.88		
CD at 1%	4.46	2.73	7.73		

^{*}Figures in parenthesis are Arc sine transformed values

Table.2 Evaluation of fungicides against tobacco black shank disease under Field condition during *kharif*, 2016

Treatments	Concentration (%)	% Disease incidence	Percent disease reduction over control	Yield (Kg/ha)			
				Green leaf	Cured leaf	TGE	
Fosetyl Al	0.1	34.92 (36.23)	54.17	6188.71	726.22	544.66	
Zineb 68% + Hexaconazole 4% WP	0.2	49.92 (36.24)	34.48	7863.02	925.06	647.54	
Cymoxanil 8% + Mancozeb 64% WP	0.2	23.02 (28.64)	69.79	9920.63	1167.13	700.28	
Trifloxystrobin 25% and Tebuconazole 50 %WG	0.1	39.68 (39.06)	47.92	6804.82	800.57	640.45	
Famoxadone 16.6%+ Cymoxanil 22.1 %	0.1	50.79 (45.48)	33.34	7848.32	923.33	738.67	
Azoxystrobin 23 EC	0.1	25.40 (30.21)	66.66	9553.20	1123.91	674.34	
Fenamidone 10% + Mancozeb 50 WG	0.2	19.05 (25.80)	75.00	10596.71	1246.67	872.67	
Metalaxyl 8% + Mancozeb 64% WP (RC)	0.2	30.95 (33.74)	59.38	8377.43	985.58	689.91	
Untreated Control	-	76.19 (60.84)	0.00	5291.01	622.47	497.98	
S.Em±		1.32	-	244.48	28.76	20.27	
CD at 5%		3.97	-	732.94	86.23	60.77	

^{*}Figures in parenthesis are Arc sine transformed values

The combi fungicides is very effective in managing black shank proved by (Karegowda 2014) as the application of Fenamidone + Mancozeb consistently retarded black shank development and increased the yield when used in combination with Mancozeb due to the reason of that being contact fungicide Mancozeb does not persist long on the stem (Suryanaraya and Rajarao 1988, Amrinder *et al.*, 2009 and Ghazanfar *et al.*, 2010) and plots that applied with longer intervals were unprotected. The above statement is also in confirmation with our findings.

The effectiveness of Fenamidone + Mancozeb is attributed to its ability to inhibit the persistence of inoculum and production of

chlamydospores and oospores on the treated portions than the Metalaxyl Mz treatments. The results of Karegowda (2014) and Dam (2015) are also in line with the present studies, as they reported that application of Fenamidone + Mancozeb highly effective for the control of black shank with. CTRI. Rajmundry tried various fungicides against Phytophthora black shank disease and found Fenamidone + Mancozeb as the best for reduction in incidence on tobacco stem and it is a promising alternative to Metalaxyl Mz. The systemic fungicide metalaxyl (Ridomil, matco 8-64), Fosetyl Al (Aliette) both as foliar spray and soil drench were found effective against Phytophthora capsici in field (Ramachandran conditions and Sarma.

1985a). Similarly Raju et al., in 2016 showed that, the Fenamidone + Mancozeb and Azoxystrobin, treated the plots of tobacco reduced the incidence of black shank on stem and increased green leaf yield, cure leaf yield and total grade equivalent relative to control plots. The new molecules of fungicides is very effective in managing black shank proved by Karegowda, 2015 as application of Fenamidone + Mancozeb consistently retarded late disease development and increased the yield parameters and the plots that applied with longer intervals were unprotected. The above statement is also in confirmation with our finding.

In conclusion, Chemical control remains the most important control measure against black shank. The control strategy is primarily preventive but in case the pathogen infects the crop, the epidemic must be stopped by using powerful formulations. more All the recommended fungicides against Phytophthora parasitica f. sp. nicotianae and were found compatible effective at their respective recommended doses. Among them, combi product Fenamidone 10% + Mancozeb 50 WG (0.1 percent) significantly reduced the black shank disease incidence in the field and achieving the higher green leaf yield, cured leaf yield and total grade equivalent over other treatments.

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