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Fungicidal Management of Stemphylium blight of Onion caused by *Stemphylium vesicarium* (Wallr.) Simmons

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Onion is an important commercial crop grown all over the world. It is attacked by many diseases which cause yield losses and result in lowering the quality and export potential of the produce. Stemphylium blight caused by *Stemphylium vesicarium* (Wallr.) Simmons is one such disease, which has become an economic threat since past few years, especially in Northern and Eastern India. In the present study, field evaluation as well as *in vitro* study was conducted for evaluating some fungicides for the management of Stemphylium blight as well as to observe their effects on yield and quality parameters of onion. The results of the *in vitro* study revealed that fluopyram + tebuconazole gave complete mycelial inhibition of test fungus at 50 ppm concentration. Results of the field experiments showed that all the treatments significantly reduced the disease severity. However, strobilurins were found to be more effective, giving more than 50% disease control over check. Keeping in view the overall performance of the fungicides, it was concluded that the combination products azoxystrobin 25% + flutriafol 25% SC and fluopyram 20% + tebuconazole 20% SC can be recommended for the management of Stemphylium blight of onion under field conditions.

Keywords: Stemphylium, triazoles, strobilurins, management, fungicides, in vitro.

Onion is the most widely cultivated species of the genus *Allium* and is one of the most important commercial crops grown all over the world. India is the second largest producer of onion in the world, but falls behind other countries in terms of productivity. Among the various reasons, diseases and pests are an important constraint in onion production. The crop is attacked by many diseases which cause yield losses and also result in deteriorating the quality and export potential of the produce. Stemphylium blight caused by *Stemphylium vesicarium* (Wallr.) Simmons is one such disease, which was not a major economic threat earlier, but has become a serious problem throughout the country since recent past, especially in Northern and Eastern India including Uttarakhand. Surveys conducted by NHRDF indicated that Stemphylium blight was more severe in the winter/summer than in the rainy season with 1.3-100 per cent incidence (Gupta *et al.*, 1994) and sometimes may even cause 100 per cent crop losses (Singh *et al.,* 1992). Leaf blight can lead to premature defoliation the crop, thereby making it more susceptible to secondary and post-harvest infections. Disease intensity is higher in seed crop than in bulb crop. Various management strategies like cultural practices, field sanitation, and biological control have been suggested. All these methods are effective only when employed well in advance as precautionary measure (Kata, 2000). But once the disease has appeared, these methods become less effective. In such situation, chemical control offers a good choice to grower for managing the disease. Chemical pesticides

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have been used since long and have the advantage of providing quick, effective and economic management of plant diseases. Therefore, the present study was undertaken to evaluate the *in vitro* and field efficacy of some new generation fungicides against Stemphylium blight of onion. Since yield and quality parameters are the main focal point of any agronomic practice, therefore whether the applied disease control measures are effective or not, is often judged by the final harvested produce. So the effect of fungicide application on the yield of the produce was also assessed.

MATERIALS AND METHOD

In vitro Study

The present investigations on *in vitro* bioassay of different fungicides were carried out using Completely Randomized Design (CRD). Evaluation of triazole and strobilurin fungicides was carried out for their efficacy to inhibit the mycelial growth of the test fungus *Stemphylium vesicarium* by "Poisoned food technique" as described by Sharvelle (1961). The triazole fungicides viz. tebuconazole (25EC), propiconazole (25EC), difenoconazole (25EC), flutriafol (25SC); fluopyram (50SC) is a novel compound belonging to a new chemical class of succinate dehydrogenase inhibitors; strobilurin fungicides viz. azoxystrobin (25SC) and combination products viz. fluopyram + tebuconazole (40SC) and azoxystrobin + flutriafol (25SC) were tested at 50 parts per million (ppm), 100 ppm and 200 ppm concentrations. Stock solution of the fungicides, each of 10000 ppm was prepared and required volume of stock solution was added to double strength PDA medium just before pouring so as to obtain the desired concentration of the "poisoned" medium. 20 ml of the poisoned medium was poured into sterile Petri plates. After solidification, the plates were then inoculated with 5 mm mycelial discs from 10 days old culture of the test fungus cut by a sterilized cork borer and incubated at 24 ± 1ºC. Three replications were maintained for each treatment. The fungus grown on un-amended PDA served as control. The radial growth of the colony was recorded after10 days and percent mycelial growth inhibition was calculated as described by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Inhibition percent
C = Growth of the test fungus in control (mm)
T = Growth of the test fungus in treatment (mm)

Field Study

Field experiment was conducted at Vegetable Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during *rabi* crop seasons in 2013-14 using Randomized Block Design (RBD) with three replications. Three foliar sprays of fungicides at 15 days interval, beginning at 45 days after transplanting were given. The treatments used were:

Observations on disease severity were recorded one week after each spray. Disease severity in field was monitored on ten selected plants per plot in three replicated plots using 0-5 scale. Percent disease index (PDI) was calculated by the following formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves} \times \text{maximum rating value}} \times 100$$

Per cent disease control (PDC) was worked out in a similar was as per cent mycelial inhibition, i.e. by subtracting the disease severity in treatment from that of check and then dividing by disease severity in check. Harvesting of each treatment was done separately and the yield and bulb size was recorded. Total yield was recorded and marketable yield was calculated by discarding those bulbs which were having bolters, or were highly deformed or rotten and of unacceptable, non-marketable quality. Grading was done on the basis of bulb size into three grades viz. A (diameter more than 55 mm), B (diameter 45-55 mm) and C (diameter less than 45 mm).

Statistical Analysis

The results of the lab studies and field experiment were statistically analyzed using the statistical package developed by GBPUAT, Pantnagar (STPR 2 and 3). The analysis of replicated data on the mycelial growth of the test fungus was done as per CRD and that of the field replicated data on disease severity and yield was done as per RBD.

RESULTS AND DISCUSSION

In vitro Study

Eight fungicides were evaluated at three different concentrations i.e. 50 ppm, 100 ppm and 200 ppm to see their effect on mycelial growth of *Stemphylium vesicarium*. Data pertaining to colony diameter and percent inhibition of radial growth presented in Table 2 revealed that all the fungicides significantly inhibited the growth of the test fungus at all tested concentrations. Fluopyram, tebuconazole and their combination product fluopyram + tebuconazole were found most effective at all the concentrations, giving 100 per cent growth inhibition, followed by propiconazole. At 50 ppm concentration (Fig 1), azoxystrobin was found less effective giving 29.20 per cent mycelial inhibition. Same trend followed at 100 and 200 ppm concentration, where highest inhibition (100%) was by fluopyram, tebuconazole and fluopyram + tebuconazole. Azoxystrobin and azoxystrobin + flutriafol were found at par giving the least per cent inhibition in all concentrations. Among the triazoles, tebuconazole provided 100 percent growth inhibition at all concentrations and was found most effective followed by propiconzole, flutriafol and difenoconazole. Fluopyram was found quite effective but its combination with azoxystrobin reduced its efficacy. It was almost at par with azoxystrobin. It showed that azoxystrobin

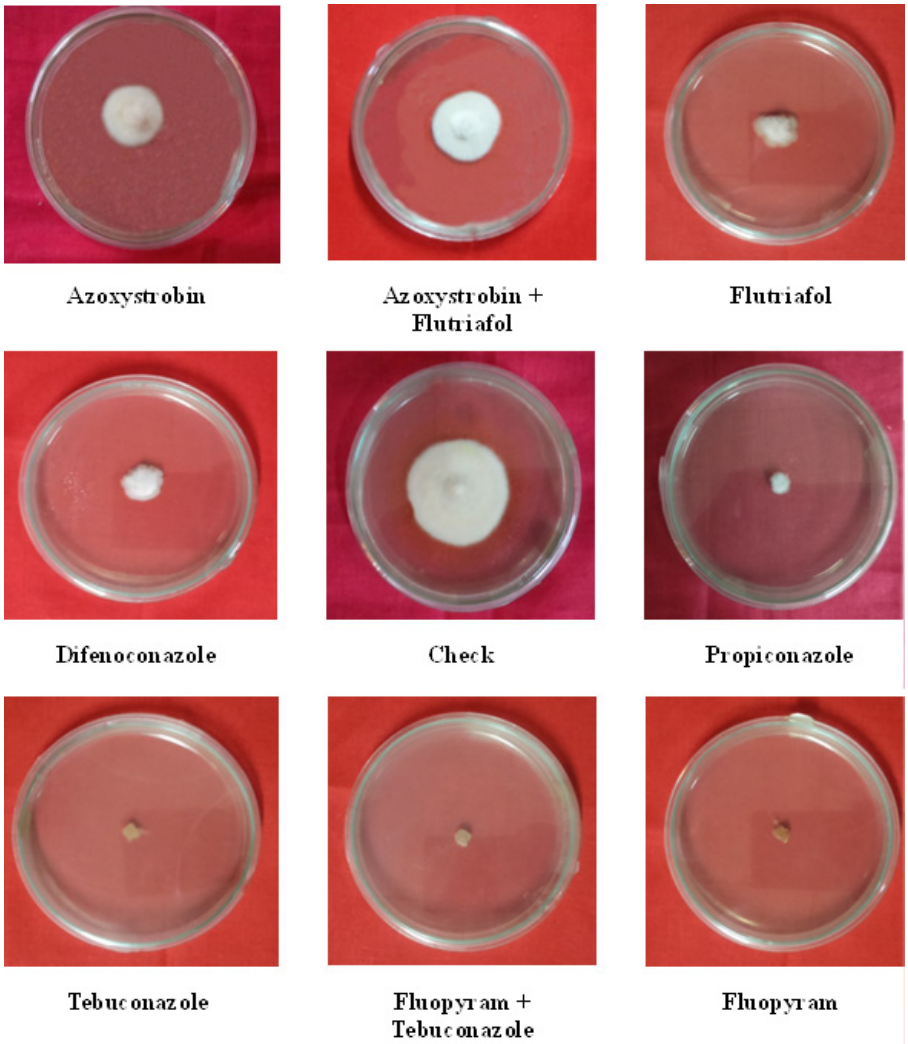

Fig. 1. Effect of fungicides on the radial growth of *Stemphylium vesicarium* at 50 ppm

and its combination azoxystrobin + flutriafol were not effective in inhibiting the mycelial growth of *Stemphylium vesicarium.* The effect of fungicide treatment as well as that of fungicide concentration and their interaction was found significant in inhibiting the mycelial growth of the test fungus.

The result of present study is in accordance with the reports by Collina *et al.* (2006) on *in vitro* sensitivity of *Stemphylium vesicarium* to various fungicides which showed that best activity on mycelial growth was obtained with anilinopyrimidines, tebuconazole, flutriafol, difenoconazole and propiconazole. Mishra and Gupta (2012) conducted *in vitro* study and found that azoxystrobin (0.1 %), propiconazole (0.1 %) were effective. Sensitivity of *S. vesicarium* to strobilurin fungicides has also been confirmed by Alberoni *et al.* (2006). However, the present study showed that azoxystrobin was less effective as compared to triazoles. The reports of triazoles being effective were supported by Mohan *et al.* (2004) who recorded the highest pathogen inhibition by triazole fungicides. Out of the 3 triazoles tested, tebuconazole was the most effective for *S. botryosum* followed by difenoconazole and hexaconazole. In the present study also, tebuconazole was found to be best, giving complete inhibition of *S. vesicarium*, followed by propiconazole and flutriafol, which confirms the superiority of triazole fungicides over others in inhibiting the growth of *S. vesicarium* and indicates that there is significant difference in the efficacy within the members of triazole group also.

Table 1. List of fungicides and their doses used for the field studies

S. No.	Fungicide	Dose (g a.i. ha^{-1})
1.	Fluopyram 50% SC	75
2.	Tebuconazole 25% EC	75
3.	Propiconazole 25% EC	125
4.	Difenoconazole 25% EC	125
5.	Azoxystrobin 25% SC	100
6.	Flutriafol 25% SC	100
7.	Fluopyram 20%SC + Tebuconazole 20% SC	150
8.	Azoxystrobin 25% SC + Flutriafol 25% SC	25

g a.i. ha^{-1} = active ingredient in grams per hectare

Table 2. Effect of different fungicides and their concentrations on the mycelial growth of *S. vesicarium*

Treatments	Colony Diameter (mm)*			Growth Inhibition (%)		
	50ppm	100ppm	200ppm	50ppm	100ppm	200ppm
Fluopyram	0	0	0	100	100	100.00
Propiconazole	8.33	6	2.67	81.75	86.86	94.16
Tebuconazole	0	0	0	100	100	100.00
Difenoconazole	18.17	17	13.17	60.22	62.77	71.17
Fluopyram+Tebuconazole	0	0	0	100	100	100.00
Azoxystrobin	32.33	28.67	25.5	29.2	37.23	44.16
Flutriafol	16.17	11.83	9.67	64.6	74.09	78.83
Azoxystrobin+Flutriafol	31.33	29	26.17	31.39	36.5	42.70
Check	45.67	45.67	45.67	nil	nil	nil
		S. Em ±		CD (p=0.05)		
Treatments		0.55		1.56		
Concentrations		0.32		0.90		
Treatment X Concentration		0.95		2.70		
CV		10.79				

*all values are mean of three replication

Field Study

The results of the trial are presented in Table 3. All the treatments were found to be significantly effective over check in reducing the disease severity. Disease severity, 45 days after transplanting (DAT), was found to be least in azoxystrobin + flutriafol (6.51 %) followed by azoxystrobin (8.79%). Azoxystrobin was found at par with fluopyram + tebuconazole. Within the treatments, least PDC was found in case of propiconazole (30.65%). At 60 DAT, per cent disease index (PDI) ranged from 26.26 to 60.35 %. Maximum disease severity was observed in check and highest PDC was offered by azoxystrobin (56.50 %). Azoxystrobin was found at par with azoxystrobin + flutriafol. At 75 DAT, fluopyram + tebuconazole was found to be most effective with lowest disease severity while propiconazole was found to be less effective. Thus, per cent disease control (PDC) was highest in fluopyram + tebuconazole (46.28%) followed by azoxystrobin (43.39%) and azoxystrobin + flutriafol (42.17%).

Ureba *et al.* (1998) found Tebuconazole effective in controlling garlic leaf spots. Bhatia and Chahal (2014) reported that Tebuconazole 25.9 EC, Propiconazole 25 EC etc are effective in managing Stemphylium blight, but in our study we observed that although triazole fungicides

Table 3. Field evaluation of fungicides for management of Stemphylium blight of onion

Treatments	Percent Disease Index*			Percent Disease Control		
	45 DAT	60 DAT	75 DAT	45 DAT	60 DAT	75 DAT
Fluopyram	10.36	32.02	48.90	46.46	46.95	38.50
Propiconazole	13.41	37.14	59.88	30.65	38.46	24.69
Tebuconazole	10.80	35.60	47.55	44.15	41.01	40.20
Difenoconazole	12.19	35.15	54.61	37.00	41.75	31.31
Fluopyram+Tebuconazole	9.49	32.51	42.71	50.96	46.14	46.28
Azoxystrobin	8.79	26.26	45.01	54.55	56.50	43.39
Flutriafol	12.28	35.68	49.25	36.51	40.88	38.05
Azoxystrobin+Flutriafol	6.51	28.02	45.98	66.34	53.58	42.17
Check	19.34	60.35	79.51	-	-	-
S.Em ±	0.47	1.79	0.66			
CD (p=0.05)	1.41	5.37	1.99			
CV	7.13	8.65	2.18			

*all values are mean of three replications, DAT= Days After Transplanting

Table 4. Effect of fungicide application on the yield and bulb size of onion

Treatments	Total Yield* ($t\ ha^{-1}$)	Marketable Yield* ($t\ ha^{-1}$)	Bulb Grades (%) A	B	C
Fluopyram	20.38	19.51	4.79	16.79	78.42
Propiconazole	21.19	20.59	2.21	19.92	77.87
Tebuconazole	23.83	22.26	7.65	24.98	67.38
Difenoconazole	22.69	22.01	5.73	18.62	75.65
Fluopyram+Tebuconazole	22.65	22.05	8.28	18.37	73.34
Azoxystrobin	21.06	19.97	2.90	23.01	74.10
Flutriafol	24.97	23.51	5.78	17.95	76.27
Azoxystrobin+Flutriafol	22.28	21.56	3.74	18.69	77.57
Check	16.32	15.98	1.95	14.50	83.55
S.Em ±	1.21	1.11			
CD (p=0.05)	3.64	3.32			
CV	9.69	9.23			

*all values are mean of three replications

offered disease control, but within the triazole group there is remarkable difference in efficacy against Stemphylium blight of onion. Flutriafol was most effective followed by tebuconazole and difenoconazole. Propiconazole was found least effective. Mohan *et al.* (2004) who recorded that out of the 3 triazoles tested, tebuconazole was the most effective for *S. botryosum* followed by difenoconazole and hexaconazole, thus showing the high degree of variation among the same group of fungicides. This study supports our findings, that there are differences in the efficacy of triazole fungicides and tebuconazole was found superior over other triazoles like propiconazole. Results of field trials by Tesfaendrias *et.al* (2012) showed that azoxystrobin + difenoconazole (Quadris top), fluopyram + pyremethanil (Luna Tranquility), difenoconazole (Inspire) were the most effective in reducing stemphylium leaf blight, which is supporting the findings of the present study that combination products especially a combination of strobilurin and triazole (azoxystrobin + flutriafol) are providing a better disease control.

Strobilurin fungicides are quinone outside inhibitors and are effective in inhibiting the germination of fungal spores. Although their effect against mycelial growth of the fungus is also documented, but inhibiting spore germination is what they do best. On the other hand, triazole fungicides inhibit ergosterol biosynthesis in pathogenic fungi. Because spores already contain ergosterol, the triazole fungicides are generally not very effective in preventing spore germination. Triazole fungicides work best by inhibiting fungi's mycelial growth (*Bradley, 2011)*. This might be the probable reason of difference in the *in vitro* and field results discussed above. Strobilurins have the novel mode of action and thus perform better under field conditions rather than providing direct mycelial inhibition *in vitro*. Moreover, strobilurin fungicides have been researched for their potential physiological effects like delayed senescence, altered amounts of plant hormones, increased activity of antioxidative enzymes which further strengthens the plants in fields and thus quite possibly provide better disease management.

Effect on Yield

All the treatments were separately harvested and their yields were recorded and bulbs were graded on the basis of size. The results presented in Table 4 shows the effect of fungicidal sprays on yield and bulb size. The data revealed that all the treatments were significantly effective over control in increasing the yield but within the treatments, all the treatments were found at par in terms of marketable yields. However, the higher total as well as marketable yields were obtained in the triazole treatments (flutriafol, tebuconazole and fluopyram + tebuconazole). The lowest total and marketable yield were obtained in case of check (16.32 and 15.98 t ha^{-1}). The highest share of A grade bulbs in the marketable yield (8.28 %) was in case of fluopyram + tebuconazole although all treatments were at par. These results are in accordance with the studies of Mohan *et al.* (2004) working on chilli and onion, who recorded that the triazole fungicides also produced the highest yields.

CONCLUSION

Laboratory studies revealed that fluopyram + tebuconazole at 50 ppm completely inhibited the radial growth of *S. vesicarium*. Under field conditions, azoxystrobin + flutriafol was found most effective in reducing the disease severity and providing better disease control. Keeping in view the overall performance of all the tested fungicides, the combination products were found to be superior in managing the disease. Therefore, azoxystrobin 25% + flutriafol 25% SC @ 25 g a.i. ha^{-1} and fluopyram 20% + tebuconazole 20% SC @150 g a.i. ha^{-1} can be recommended for the management of Stemphylium blight of onion under field conditions.

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