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EFFICACY OF STROBILURIN AND TRIAZOLE FUNGICIDES FOR THE MANAGEMENT OF POWDERY MILDEW (*Sphaerotheca fuliginea*) IN SUMMER SQUASH

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ABSTRACT

Summer squash (*Cucurbita pepo* L.) is cultivated extensively in temperate and subtropical regions. Among the various challenges faced by this crop, powdery mildew stands out as one of the most damaging diseases affecting cucurbits, including summer squash. This disease inflicts significant damage on crops grown in both open-field conditions and protected environments. In the present study, we evaluated various systemic and non-systemic fungicides for the management of powdery mildew in summer squash, both under *in vitro* and field conditions. In our search for new alternatives to carbendazim, difenoconazole, at a concentration of 100 ppm, resulted in only 1.75% conidial germination. This was followed by hexaconazole, which achieved a germination rate of 2.53%, compared to a control group exhibiting 48.32% germination. Hexaconazole also yielded a germ tube length of 2.16 µm, while difenoconazole alone produced 2.91 µm, and the combination of difenoconazole + azoxystrobin resulted in 3.44 µm germ tube length. Under field conditions over two consecutive years, four applications of azoxystrobin at 0.1% demonstrated the highest efficacy, resulting in a disease index of 14.33% and an 81.71% reduction in disease severity compared to the untreated control, which had a disease index of 78.33%. Hexaconazole (0.05%) and difenoconazole (0.03%) were also effective, reducing the disease index to 19.67% and 22.33%, respectively, corresponding to reductions in disease severity of 74.89% and 71.49%. Notably, these fungicides significantly increased crop yield under field conditions over the two-year study period.

Key words: *Cucurbita pepo*, Fungicides, Management, *Systemic*

Summer squash (*Cucurbita pepo* L.) is an important vegetable crop grown in both the hills and plains of India. In the mid-hills of Himachal Pradesh, it is grown as an off-season vegetable from March to August. The fruits are subsequently transported to markets in the northern plains, where they fetch lucrative prices. However, during the month of June, the crop is severely affected by powdery mildew, caused by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (formerly *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.) and *Golovinomyces cichoracearum* (DC.) V. P. Heluta (formerly *Erysiphe cichoracearum* (DC.) V. P. Gelyuta). Typically, *P. xanthii* is most commonly associated with cucurbit powdery mildew in tropical or subtropical regions and greenhouse crops, while *G. cichoracearum* is more prevalent in temperate regions and field crops (Caligiore-Gei *et al.*, 2022). However, the occurrence of *S. fuliginea* (now *P. xanthii*) has been reported in Himachal Pradesh, India, where it has been associated with high disease severity ranging from 30% to 77% (Bharmera and Gupta, 2018; Bharmera *et al.*, 2021).

Foliar sprays of fungicides represent a common strategy for managing powdery mildew in cucurbits; however, the options available are very limited. This could be due to the emergence of more aggressive pathogen populations and/or the development of fungicide resistance in powdery mildew pathogens (Goswami and Thind, 2012). Current literature provides limited information on the efficacy of new-generation fungicides, particularly strobilurins and ergosterol biosynthetic inhibitors (EBIs), in controlling powdery mildew in cucurbits (Bharmera and Gupta, 2018). The availability of a diverse range of effective fungicides against powdery mildew could facilitate the development of combination treatments, thereby mitigating the risk of resistance development in the pathogen. In light of these considerations, the present study aimed to evaluate the efficacy of various strobilurin and triazole fungicides, both individually and in combination, against powdery mildew. This evaluation was conducted in comparison to standard mildewcides, including wettable sulfur, dinocap, and carbendazim, under both *in vitro* and field conditions.

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MATERIALS AND METHODS

Twelve different systemic and non-systemic fungicides were evaluated for their efficacy against *S. fuliginea* under both *in vitro* and field conditions. These included Amistar 23 SC (Azoxystrobin 25% SC), Amistar Top SC (Azoxystrobin 20% + Difenoconazole 12.5%), Cabrio Top 60 WG (Pyraclostrobin 5% + Metiram 55%), Nativo (Trifloxystrobin 50% + Tebuconazole 25% WG), Luna (Fluopyram 17.7% + Tebuconazole 17.7% SC), Kresoxymethyl 44.3 SC (Kresoxymethyl), Contaf 5% EC (Hexaconazole), Score 25% EC (Difenoconazole), Taspa (Propiconazole 13.9% + Difenoconazole 13.9% EC), Sulfex 80 WP (Wettable Powder), and Karathane 48% EC (Dinocap), with Bavistin 50% WP (Carbendazim) serving as the standard fungicide.

Effect of fungicides on conidial germination and germ tube elongation under *in vitro* conditions

The efficacy of twelve different fungicides was evaluated against the powdery mildew pathogen using the slide-germination method (Thind *et al.*, 1996). An atomizer was used to spray the required concentrations of fungicides onto clean, grease-free, sterilized glass slides, which were then allowed to dry. Powdery mildew conidia from freshly sporulating pustules (Fig. 1a) were gently dusted onto the slides. To maintain the high humidity necessary for conidial germination, approximately 5 µl of sterilized distilled water was placed on the corners of the dusted slides using a micropipette (Thind *et al.*, 1996). The slides were then positioned on glass-rod triangles within moist chambers and incubated at 25 ± 2°C. For the control treatment, conidia were dusted onto slides that had been sprayed with sterilized distilled water. Observations on both the germination of conidia and the length of the germ tubes were recorded after 72 hours of incubation.

Evaluation of fungicides under *in vivo* conditions

Fungicides at selected concentrations from the *in vitro* screening were further assessed under field conditions. The experiments were conducted under natural epiphytotic conditions at the experimental farm of the Department of Plant Pathology of the university. Field trials were organized in a randomized block design (RBD) with three replications during two consecutive crop seasons, 2017 and 2018. Seeds of the susceptible variety ‘AG’ were sown in pits with a spacing of 90 x 90 cm for rows and 50 x 50 cm for plants. Twelve plants per replication were maintained. The first spray of fungicides was applied when the disease initiated in the last week of May, followed by three additional sprays at 10-day intervals. Disease severity was assessed seven days after the last spray using a 0-5 scale (0 = no infection, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100%). The Disease Index (%) was calculated using the following formula:

Disease index (%) = {Sum of all disease ratings/(Total number of disease ratings x Maximum disease grade)} x 100

Fruit yield data were recorded for each harvest and totalled for each plot to calculate the yield per plot. The data for disease index and fruit yield from both crop seasons were pooled. Disease reduction was calculated using the formula:

Disease reduction (%)= {(C-T)/C}x 100.

where C is the control value and T is the treatment value. Similarly, the increase in yield (%) was also calculated.

Statistical analysis

Data were analyzed using one-way or two-way Analysis of Variance (ANOVA), depending on the experimental design (completely randomized design [CRD] or randomized block design [RBD]), after applying the appropriate transformations. Different treatments were compared for all possible pairwise comparisons of means within a factor using an equivalent of multiple t-tests. The Least Significant Difference (LSD) test was employed to determine significant differences between treatments. The critical difference (CD) in the LSD test was calculated using the following formula:

$$CD = t_{0.05}(error\ d.f)\sqrt{\frac{2(MSS)_{error}}{n}}$$

where t is the critical value from Student’s t-distribution at a 95% confidence level, df represents the degrees of freedom, MSS is the mean square error, and n is the number of observations.

RESULTS AND DISCUSSION

Effect of fungicides on conidial germination and germ tube elongation under *in vitro* conditions

Compared to the control group, all fungicides significantly reduced the germination of conidia (Table 1). The conidia can germinate by producing either a forked germ tube (Fig. 2a) or a simple germ tube (Fig. 2b). Overall, there was a decrease in conidial germination with increase in fungicide concentration (Fig. 3). While carbendazim proved to be the most effective at all concentrations, completely inhibiting conidial germination, the emergence of benzimidazole resistance in *S. fuliginea* (now *P. xanthii*) has been

Table 1. Effect of fungicides on germination of conidia and germ tube elongation

Treatment	Conidial germination (%) Concentration (ppm)			Overall mean	Germtube length (µm) Concentration (ppm)			Overall mean
	25	50	100		25	50	100	
Azoxystrobin 25% SC	18.20 (25.22)	12.71 (20.86)	7.92 (16.33)	12.94 (20.80)	17.90	13.62	6.82	12.78
Azoxystrobin 20% + difenoconazole 12.5% w/w	15.11 (22.85)	9.04 (17.48)	3.98 (11.48)	9.37 (17.27)	13.61	8.84	3.44	8.63
Pyraclostrobin 5% + metiram 55% WG	16.16 (23.69)	10.98 (19.33)	5.93 (14.07)	11.02 (19.03)	15.74	9.04	5.20	9.99
Trioxystrobin 50 % + tebuconazole 25 %	25.89 (30.57)	19.81 (26.42)	12.46 (20.65)	19.39 (25.88)	28.21	20.20	15.46	21.29
Fluopyram 17.7 + tebuconazole 17.7 SC	20.15 (26.66)	14.07 (22.00)	9.07 (17.51)	14.43 (22.06)	19.09	14.86	10.84	14.93
Kresoxymethyl 44.3 SC	23.10 (28.71)	16.08 (23.60)	10.86 (19.23)	16.68 (23.85)	25.81	20.50	14.84	20.38
Hexaconazole 5% EC	12.89 (21.03)	7.94 (16.34)	2.53 (9.13)	7.79 (15.50)	10.52	6.70	2.16	6.46
Propiconazole 13.9 % + difenoconazole 13.9 %	21.42 (27.55)	14.27 (22.19)	9.88 (18.31)	15.91 (22.68)	21.41	16.24	12.59	16.75
Difenoconazole 25 % EC	9.89 (18.32)	5.72 (13.82)	1.75 (7.49)	5.79 (13.21)	7.99	5.29	2.91	5.39
Carbendazim 50 % WP	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00	0.00
Dinocap[b] 48 % EC	2.69 (9.39)	1.83 (7.73)	0.00 (0.00)	1.51 (5.71)	4.61	1.77	0.00	2.13
Wettable Sulfur[a] 80 WP	6.93 (15.24)	2.98 (9.92)	0.00 (0.00)	3.31 (8.39)	6.75	3.39	0.00	3.38
Control	51.84 (46.04)	-	-	51.84 (46.04)	31.24	-	-	31.24
CD ($p \leq 0.05$)	Fungicides (F) = (0.61) Concentration (C) = (0.29) F × C = (1.05)				Fungicides (F) = (0.48) Concentration (C) = (0.23) F × C = (0.82)			

Figure in parentheses are arcsine transformed values
a: Tested at 250, 500 and 1000 ppm
b: Tested at 100, 200 and 500 ppm

Fig. 1a. Initial symptoms of powdery mildew on summer squash leaf; b. Completely infected summer squash plant

Fig. 2a. Conidium and germinated conidium with forked germtube; b. Conidium showing simple germtube

widely reported globally. This highlights the need for alternative, effective fungicides to combat powdery mildew in cucurbits (Vielba-Fernández *et al.*, 2020). Similarly, dinocap at 500 ppm and wettable sulfur at 1000 ppm also achieved complete inhibition of conidial germination. However, cucurbits are sensitive to sulfur, and high doses of sulfur fungicides should be avoided in these crops. Difenoconazole, at 100 ppm, resulted in only 1.75% conidial germination, while hexaconazole yielded 2.53%, compared to 48.32% germination in the control group. The combination of difenoconazole and azoxystrobin at 100 ppm was also highly effective, resulting in 3.98% conidial germination. The combination of difenoconazole and propiconazole at 100 ppm led to a 9.88% germination rate. Among the strobilurin fungicides, besides the azoxystrobin and difenoconazole combination, pyraclostrobin and metiram at 100 ppm also proved effective, with a germination rate of 5.93%. This was followed by azoxystrobin, kresoxymethyl, and trioxystrobin combined with tebuconazole, which showed germination rates of 7.92%, 10.86%, and 12.46%, respectively, at 100 ppm. .

The data regarding germ tube length revealed that all tested fungicides significantly reduced germ tube length compared to the control (Table 1). A clear correlation was observed between increased fungicide concentrations and decreased germ tube length (Fig. 4). Consistent with the patterns seen in conidial germination, a similar trend emerged for germ tube length. The minimum germ tube length recorded was 1.77 µm with dinocap at 500 ppm, followed by lengths of 2.16 µm and 2.91 µm for hexaconazole and difenoconazole, respectively, both at 100 ppm. In contrast, the control exhibited a germ tube length of 31.24 µm, with these treatments showing statistically comparable effects. The difenoconazole + azoxystrobin combination at 100 ppm proved to be highly effective, resulting in a germ tube length of 3.44 µm. On the other hand, the combination of difenoconazole with propiconazole at 100 ppm yielded a longer germ tube length of 12.59 µm. Other strobilurin fungicides, specifically pyraclostrobin combined with metiram at 100 ppm and azoxystrobin at 100 ppm, also demonstrated efficacy, resulting in germ tube lengths of 5.20 µm and 6.82 µm, respectively. Furthermore, the combination of tebuconazole with fluopyram produced a germ tube length of 10.84 µm, while the combination of tebuconazole + trioxystrobin at 100 ppm resulted in a length of 15.46 µm. Overall, the mean data indicated that carbendazim was the most effective treatment, followed by dinocap (2.13 µm), wettable sulfur (3.38 µm), and difenoconazole (5.39 µm). In contrast, the combination of trioxystrobin and tebuconazole produced the least effective result at 21.29 µm, although this was still superior to the control. Generally, while combination fungicides tend to have lower concentrations of individual components and, consequently, lower efficacy compared to single fungicides, they are preferred to mitigate the risk of developing fungicide-resistant strains among fungal pathogens.

Evaluation of fungicides under *in vivo* conditions

The severity of powdery mildew disease was significantly reduced by foliar applications of fungicides under field conditions over two consecutive years (Table 2; Fig. 5). Among the treatments, azoxystrobin demonstrated the highest efficacy in reducing powdery mildew, achieving a disease index of 14.33 and a corresponding disease reduction of 81.71% compared to the control, which exhibited a disease index of 78.33%. Following azoxystrobin, carbendazim, hexaconazole, difenoconazole, pyraclostrobin + metiram, and the combination of azoxystrobin + difenoconazole yielded disease indices of 17.67, 19.67, 22.33, 24.33, and 26.00, respectively, with disease reductions of 77.44%, 74.89%, 71.49%, 68.94%, and 66.81%. Propiconazole + difenoconazole and tebuconazole + fluopyram were also effective with disease indices of 27.33 and 28.67, corresponding to reductions of 65.11% and 63.40%, respectively. Kresoxymethyl showed limited effectiveness, with a disease index of 33.67 and a 57.02% reduction in disease severity. In addition to disease management, foliar applications of fungicides resulted in significantly higher fruit yield compared to the control group (2.35 kg/ plot) (Table 2; Fig. 5). In plots treated with azoxystrobin, the highest fruit yield was recorded at 5.32 kg per plot, representing a remarkable increase of 55.83%. This was closely followed by carbendazim (5.07 kg per plot) and hexaconazole (4.60 kg per plot), both of which demonstrated statistically significant differences and increases of 53.65% and 48.91%, respectively, over the control. Difenoconazole (4.37 kg per plot) and pyraclostrobin + metiram (4.15 kg per plot) were the next most effective treatments, resulting in increases of 46.10% and 43.37% in yield, respectively. The lowest fruit yield of 3.02 kg per plot was observed in the plots treated with kresoxymethyl.

The mid-hill regions of Solan district in Himachal Pradesh are renowned for off-season cultivation of various vegetables, including cucurbitaceous crops. However, these crops are frequently afflicted by powdery mildew disease. The association of *Sphaerotheca fuliginea* (now reclassified as *Podosphaera xanthii*) with this disease in cucumber and summer squash has been documented (Gupta *et al.*, 2001; Bharmera *et al.*, 2021). Keeping in view the widespread reports of benzimidazole resistance in *S. fuliginea* (Vielba-Fernández *et al.*, 2020), there is a pressing need for alternative fungicides to effectively manage powdery

Table 2. Effect of foliar spray applications of fungicides on powdery mildew disease and fruit yield of summer squash under field conditions

Treatment	Concen-tration (%)	Disease index (%)			Disease reduction over cotrol (%)	Yield (kg / plot)			Increase in yield over control (%)
		2017	2018	Pooled		2017	2018	Pooled	
Azoxystrobin 25 % SC	0.1	13.33 (21.40)	15.33 (22.97)	14.33 (22.20)	81.71	5.43	5.20	5.32	55.83
Azoxystrobin 20% + difenoconazole 12.5 % w/w	0.06	24.00 (29.31)	28.00 (31.93)	26.00 (30.63)	66.81	3.93	3.83	3.88	39.43
Carbendazim 50 % WP	0.1	16.67 (24.08)	18.67 (25.54)	17.67 (24.82)	77.44	5.10	5.03	5.07	53.65
Pyraclostrobin 5 % + metiram 55 % WG	0.1	23.33 (28.87)	25.33 (30.20)	24.33 (29.54)	68.94	4.20	4.10	4.15	43.37
Trioxystrobin 50 % + tebuconazole 25 % WG	0.1	29.33 (32.77)	31.33 (34.02)	30.33 (33.41)	61.28	3.27	3.17	3.22	27.02
Fluopyram 17.7 + tebuconazole 17.7 SC	0.05	28.67 (32.35)	28.67 (32.36)	28.67 (32.35)	63.4	3.43	3.40	3.42	31.29
Kresoxymethyl 44.3 SC	0.1	32.67 (34.84)	34.67 (36.05)	33.67 (35.45)	57.02	3.07	2.97	3.02	22.19
Hexaconazole 5 % EC	0.05	18.67 (25.54)	20.67 (27.01)	19.67 (26.30)	74.89	4.67	4.53	4.60	48.91
Propiconazole 13.9 % + difenoconazole 13.9 % EC	0.1	26.67 (31.01)	28.00 (31.93)	27.33 (31.49)	65.11	3.63	3.50	3.57	34.17
Wettable sulphur	0.2	26.00 (30.63)	26.67 (31.01)	26.33 (30.86)	66.39	3.77	3.60	3.68	36.14
Difenoconazole 25 % EC	0.03	21.33 (27.46)	23.33 (28.86)	22.33 (28.17)	71.49	4.43	4.30	4.37	46.1
Dinocap 48 % EC	0.05	28.52 (32.00)	30.24 (33.12)	29.38 (32.78)	62.49	2.85	3.47	3.16	25.63
Control	Nil	75.33 (60.27)	81.33 (64.63)	78.33 (62.38)	-	2.40	2.30	2.35	-
CD (*p*≤*0.05*)		(2.93)	(3.52)	(2.81)		(0.33)	(0.19)	(0.18)	

Figure in parentheses are arcsine transformed values

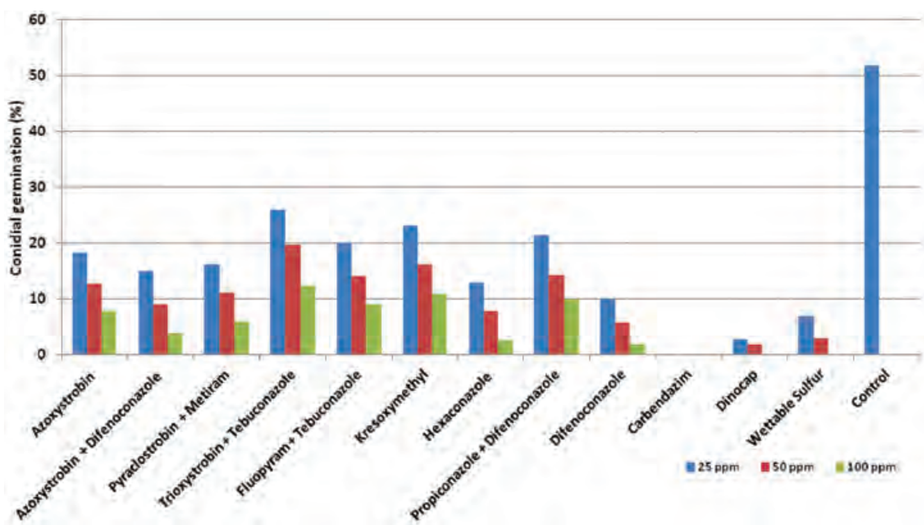

Fig. 3. Effect of different fungicide concentrations on conidial germination of *Podosphaera xanthii*

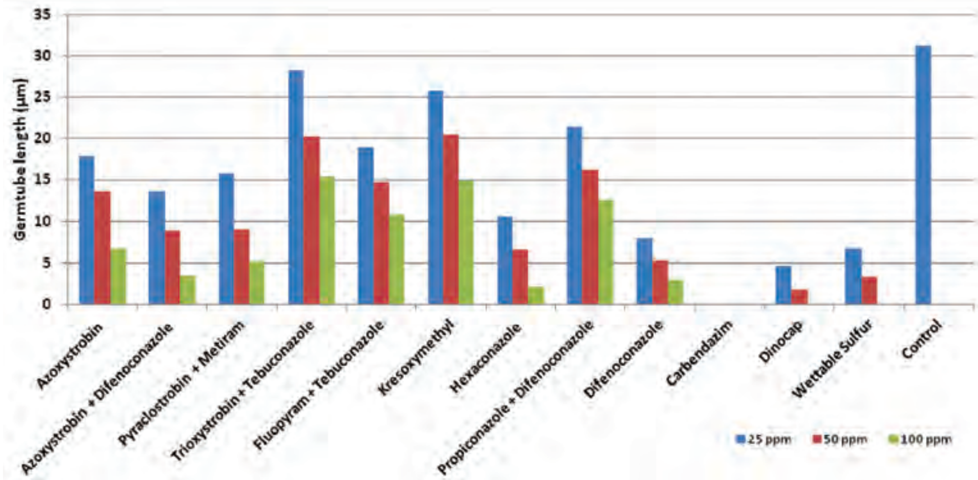

Fig. 4. Effect of different fungicide concentrations on conidial germ tube of Podosphaera xanthii

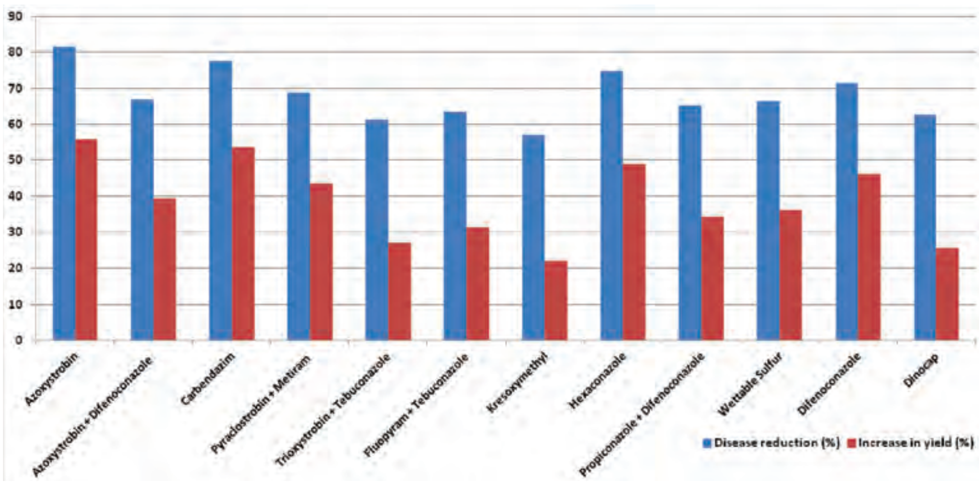

Fig. 5. Effect of different fungicides on reduction of powdery mildew and increase in yield over control in summer squash under field conditions

mildew in cucurbits.

Cucurbits are sensitive to sulfur, and the application of sulfur-based fungicides, such as dinocap and wettable sulfur, should be avoided at high doses. Therefore, the present study aimed to identify effective fungicides for managing powdery mildew. We found that difenoconazole and azoxystrobin, both individually and in combination, along with hexaconazole, were effective in controlling the disease under both *in vitro* and *in vivo* conditions. Additionally, these treatments contributed to increased yield in field trials conducted over two consecutive years.

The inhibitory effects of hexaconazole and difenoconazole against powdery mildew pathogens in various crops have been documented (Shivanna *et al.*, 2006; Gupta and Kumar, 2008). While the efficacy of propiconazole and azoxystrobin in controlling powdery mildew in summer squash has been noted—demonstrating a disease severity of 1.5% compared to 31.0% in the control (Goswami and Thind, 2012)—these studies did not assess the impact of these fungicides on conidial germination. The present investigations clearly indicate the effectiveness of azoxystrobin and triazole fungicides in reducing conidial germination, managing powdery mildew severity, and enhancing fruit yields under field conditions. Azoxystrobin is a potent inhibitor of spore germination and the early stages of fungal development. Its unique properties allow it to diffuse through the leaf tissue (the application site) and reach the vascular system, where it moves acropetally through the transpiration stream, providing excellent protection. Moreover, there is increasing evidence of its direct influence on host physiology (Koehle *et al.*, 2002). Strobilurins exhibit a “greening effect,” which refers to delayed leaf senescence, ultimately leading to enhanced biomass and yield. Triazoles, on the other hand, demonstrate translaminar movement within the leaf and can also translocate through xylem vessels from the lower to the upper portions of the plant. To mitigate the risk of developing resistance, it is essential to employ fungicides with different modes of action and movement characteristics in a rotational strategy.

CONCLUSION

In conclusion, this study highlights the significant potential of various fungicides, particularly azoxystrobin and triazole-based compounds such as hexaconazole and difenoconazole, in effectively managing powdery mildew in cucurbitaceous crops. The findings demonstrate that these fungicides not only reduce conidial germination but also decrease disease severity and enhance fruit yields under field conditions. The superior performance of azoxystrobin, attributed to its ability to inhibit spore germination and translocate effectively within the plant, highlights its role as a critical component in disease management strategies.

Furthermore, the research emphasizes the importance of integrating fungicides with diverse modes of action and movement characteristics in a rotational application approach. This strategy is essential to mitigate the risk of developing resistance among powdery mildew pathogens, ensuring sustainable and effective control measures for cucurbitaceous crops. The results advocate for the adoption of these fungicides in off-season vegetable cultivation in regions prone to powdery mildew, contributing to improved agricultural productivity and economic viability for farmers.

Authors' contribution

Conceptualization and designing of the research work (SKG, MS), Execution of field/lab experiments and data collection (PB), Analysis of data and interpretation (PB, MS), and Preparation of manuscript (PB, MS and DS).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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