Biological Forum – An International Journal **13(2): 367-373(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In vitro Evaluation of various Fungicides against Spore Germination Inhibition of *Erysiphe cichoracearum* DC. in bhendi

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(Received 09 April 2021, Accepted 14 June, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Bhendi powdery mildew caused by *Erysiphe cichoracearum* DC. affects all the stages of the plant growth by causing premature defoliation and resulting 17.0 to 86.6 per cent yield loss in bhendi. Looking into significance of disease various non- systemic, systemic and combi product fungicides were evaluated *in vitro*for their efficacy against *E. cichoracearum* by spore germination technique. Results revealed, among non-systemic, systemic fungicides evaluated, irrespective of concentrations tested, wettable sulphur, difenconazole recorded maximum mean spore germination inhibition of 78.68, 86.06 per cent, respectively and least spore germination inhibition (43.03 %, 68.07 %) was noticed inthiram and carbendazim. Among combi fungicides evaluated, tebuconazole 50 % + trifloxystrobin 25 % recorded maximum mean per cent inhibition of spore germination (88.06 %) and least mean per cent spore germination inhibition (40.49 %) was recorded in copper oxy chloride 14 % + copper hydroxide 14 %.

Keywords: Powdery mildew, *Erysiphe cichoracearum*, *in vitro*, spore germination technique, non- systemic, systemic and combi product fungicides.

INTRODUCTION

Plant diseases are one of the major constraints in crop production with drastic losses in the quality and quantity of the yield. Powdery mildew of bhendi caused by *Erysiphe cichoracearum* DC. has become a major limiting factor to get the economic production in North Karnataka (Siddappa *et al*., 2014). The disease is wide spread and most destructive to the crop under favourable conditions. Disease affects all the stages of the plant growth by causing premature defoliation and resulting 17.0 to 86.6 per cent yield loss in bhendi (Sridhar and Sinha, 1989). Though the plant disease management starts with cultural, mechanical, physical and biological methods but practically the chemical methods are in predominant practice to reduce the outbreak of diseases. The management of diseases by chemical means demands judicial usage accordingly to the kind of organism involved and its severity (Waychal *et al*., 2018). Hence, to know the field efficacy of any new fungicide molecules, there is need to test chemical under laboratory condition which provides useful and preliminary information regarding efficacy of fungicides against pathogen within a shortest period of time. Therefore, it serves as guide for field testing. Hence, various non- systemic, systemic and combi product fungicides were evaluated under *in vitro* conditions for their efficacy against *E. cichoracearum* by spore germination technique.

MATERIALS AND METHOD

Systemic, non-systemic and combi product fungicides were evaluated under *in vitro* by spore germination technique against *E. cichoracearum* during *rabi* 2019-20 at department of Plant Pathology, College of Agriculture, Raichur. Required concentrations of fungicides were prepared by dissolving known quantity of fungicides in sterile water separately under aseptic conditions. The conidial suspension was prepared separately in sterile water and adjusted to 4 $\times 10^3$ conidia /ml using haemocytometer. Then a drop of conidial suspension was mixed with one drop of fungicidal solution in a cavity slideto achieve the required concentration of systemic fungicides (0.05, 0.1 and 0.20 %), non-systemic and combi product fungicides (0.1, 0.2 and 0.3 %). In each treatment three replications were maintained. Treated cavity slides were kept in moist chamber and incubated at room temperature (25 ± 1°C) for 24 hours. Effect of fungicides and their concentrations on the germination of conidia was observed after 24 hours, under microscope at 40 X magnification. A control treatment was maintained with distilled water. Per cent conidial germination was calculated by the following formula.

$$\text{Per cent germination} = \frac{A}{B} \times 100$$

Where,

A = Number of conidia germinated

B = Number of conidia observed

Per cent inhibition over the control was calculated by using the formula given by Vincent (1947).

$$\text{Percent inhibition of spore germination (I)} = \frac{C-T}{C} \times 100$$

Where,

I = Per cent inhibition of spore germination

C = Germination of conidia in control.

T = Germination of conidia in treatment.

RESULTS AND DISCUSSION

All the fungicides evaluated were found significantly superior over the control with respect to spore germination inhibition. Among the various non-systemic evaluated, wettable sulphur was found to be most effective at all three concentrations with highest spore inhibition of 81.58 per cent at 0.3 per cent concentration and mean spore germination inhibition of 78.68 per cent (Table 1), which was significantly superior over other treatments because it creates a plant protective coating on the surface of the plant that prevents the spore germination. The next best fungicides were mancozeb (75.43 %) which reacts and inactivates the sulfhydral groups of amino acids and enzymes within fungal cells resulting in disruption of lipid metabolism, respiration and production of adenosine phosphate, propineb (71.39 %) which interferes at different locations in the metabolism of the fungi on several points of the respiration chain, in the metabolism of carbohydrates and proteins, in the cell membranes. This multi-site mode of action of propineb prevents development of resistance in the fungi and dinocap (67.28 %), which acts as uncoupler of oxidative phosphorylation, upsetting the electrochemical balance of the fungi cell and preventing the formation of energy rich ATP, affects the respiration and cell wall formation of fungi at the concentration of 0.3 per cent with mean spore germination inhibition of 69.85, 63.76 and 60.51 per cent, respectively. The next best treatments were chlorothalonil (65.21 %), zineb (62.12 %) at 0.3 per cent concentration with mean spore germination inhibition of 53.67 and 50.80 per cent, respectively (Fig. 1). Lowest spore germination inhibition was noticed in fungicides thiram (53.58 %) followed by copper oxychloride (56.48 %) and cuprous oxide (57.28 %) at the same concentration with the mean spore germination inhibition of 43.03, 41.39 and 40.80 per cent, respectively. However, all the fungicides showed the same trend of spore germination inhibition at lower concentrations (0.1 and 0.2 %) also.

The results obtained in the present investigations are in fine tune with the findings of Aswathanarayana (2003), who reported karathane and wettable sulphur as effective in complete inhibition of conidial germination at 0.3 per cent against grape powdery mildew. Ramesh(2011) found that maximum inhibition of conidial germination of *L. taurica* was recorded in wettable sulphur (85.71 %). Amaresh *et al*., (2013) observed that among various non-systemic fungicides evaluated against *E. cichoracearum*, mancozeb and wettable sulphur recorded the maximum mean per cent inhibition of conidial germination of 95.55 and 91.53 per cent respectively. Mansukhbhai *et al.,* (2020) reported that among the various non-systemic fungicides evaluated against *E. polygoni*, significantly highest mean spore germination inhibition (78.80 %) was observed in wettable sulphur and least effective fungicide was zineb (33.14 %).

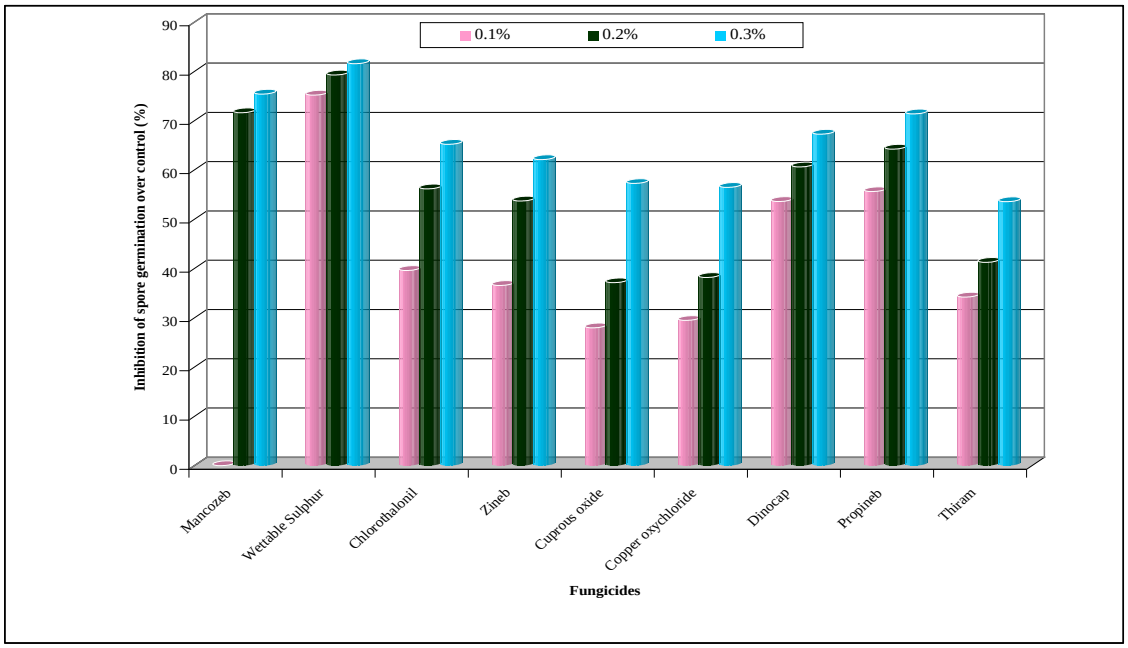

Fig. 1. *In vitro* efficacy of non-systemic fungicides against *Erysiphe cichoracearum* by spore germination technique.

Table 1: *In vitro* efficacy of non-systemic fungicides against *Erysiphe cichoracearum* by spore germination technique.

Sr. No.	Fungicide	Inhibition of spore germination over control (%)			
		Concentration (%)			Mean
		0.1	0.2	0.3	
1.	Mancozeb	62.48* (52.23)**	71.65 (57.83)	75.43 (60.29)	**69.85 (56.70)**
2.	Wettable Sulphur	75.20 (61.48)	79.28 (63.64)	81.58 (64.59)	**78.68 (62.50)**
3.	Chlorothalonil	39.62 (39.01)	56.20 (48.56)	65.21 (53.86)	**53.67 (47.10)**
4.	Zineb	36.58 (37.21)	53.72 (47.13)	62.12 (52.01)	**50.80 (45.46)**
5.	Cuprous oxide	27.98 (31.93)	37.14 (37.55)	57.28 (49.19)	**40.80 (39.70)**
6.	Copper oxychloride	29.48 (32.88)	38.21 (38.18)	56.48 (48.72)	**41.39 (40.04)**
7.	Dinocap	53.63 (47.08)	60.64 (51.14)	67.28 (55.11)	**60.51 (51.07)**
8.	Propineb	55.63 (48.23)	64.26 (53.29)	71.39 (57.66)	**63.76 (52.99)**
9.	Thiram	34.23 (35.81)	41.28 (40.56)	53.58 (47.05)	**43.03 (40.99)**
Mean		**46.09 (42.76)**	**55.82 (48.34)**	**65.59 (54.08)**	
			S.Em. (±)		**C.D. at 1 %**
Fungicides (F)			0.19		0.72
Concentration (C)			0.09		0.31
F×C			0.33		1.24

*Mean of three replications, **Angular transformed value

Among the ten systemic fungicides evaluated at three different concentrations (0.05 %, 0.10 % and 0.20 %), maximum inhibition of spore germination was observed in tebuconazole (80.95 %) at 0.05 per cent concentration which was on par with difenconazole which recorded spore germination inhibition of 79.75 per cent followed by hexaconazole (78.80 %), propiconazole (78.82 %) and penconazole (77.63 %) which were on par with each other. Next best treatments were myclobutanil and tolfenpyrad which recorded spore germination inhibition of 76.08 and 75.23 per cent and were on par with each other followed by thiophanate methyl (68.28 %) and azoxystrobin (66.40 %). Least inhibition was observed in carbendazim *i.e.*, 62.94 per cent at 0.05 per cent concentration (Table 2).

Table 2: *In vitro* efficacy of systemic fungicides against *Erysiphecichoracearum* by spore germination technique.

Sr. No.	Fungicide	Inhibition of spore germination over control (%)			
		Concentration (%)			Mean
		0.05	0.1	0.2	
1.	Azoxystrobin	66.40 * (54.57)**	76.12 (60.74)	79.24 (62.89)	**73.92 (59.29)**
2.	Carbendazim	62.94 (52.49)	68.05 (55.58)	73.23 (58.84)	**68.07 (55.59)**
3.	Difenconazole	79.75 (63.25)	87.93 (69.67)	90.51 (72.05)	**86.06 (68.07)**
4.	Hexaconazole	78.80 (62.58)	83.28 (65.86)	87.32 (69.13)	**83.13 (65.75)**
5.	Propiconazole	78.82 (62.59)	87.52 (69.31)	89.78 (71.35)	**85.37 (67.51)**
6.	Penconazole	77.63 (61.77)	81.48 (64.51)	85.51 (67.62)	**81.54 (64.55)**
7.	Tebuconazole	80.95 (64.12)	86.58 (68.51)	89.48 (71.07)	**85.67 (67.76)**
8.	Thiophanate methyl	68.28 (55.72)	73.48 (59.00)	78.48 (62.36)	**73.41 (58.96)**
9.	Myclobutanil	76.08 (60.71)	81.34 (64.40)	85.24 (67.40)	**80.88 (64.07)**
10.	Tolfenpyrad	75.23 (60.15)	79.05 (62.76)	83.28 (65.86)	**79.18 (62.85)**
	Mean	**74.48 (59.66)**	**80.48 (63.78)**	**84.20 (66.58)**	
			S.Em. (±)		**C.D. at 1 %**
Fungicides (F)			0.28		1.06
Concentration (C)			0.13		0.47
F×C			0.49		1.83

*Mean of three replications, **Angular transformed value

At 0.1 per cent concentration maximum inhibition of spore germination was observed in difenconazole (87.93 %) which is sterol demethylation inhibitor which prevents the development of fungus by inhibiting cell membrane ergosterol biosynthesis (Nene and Thapliyal, 2002) which was on par with propiconazole (87.52 %) which is demethylation inhibiting fungicide, which binds and inhibits the 14 alpha demethylase enzyme from demethylating a precursor to ergosterol followed by tebuconazole (86.58 %), hexaconazole (83.28 %) and penconazole (81.48 %) which acts on fungal pathogen during penetration and haustoria formation and stops the development of fungi by interfering with biosynthesis of sterols in cell membrane. Next best treatments were myclobutanil and tolfenpyrad which recorded spore germination inhibition of 81.34 and 79.05 per cent followed by azoxystrobin (76.12 %) and thiophanate methyl (73.48 %) and least inhibition (68.05 %) was observed in carbendazim (Fig. 2).

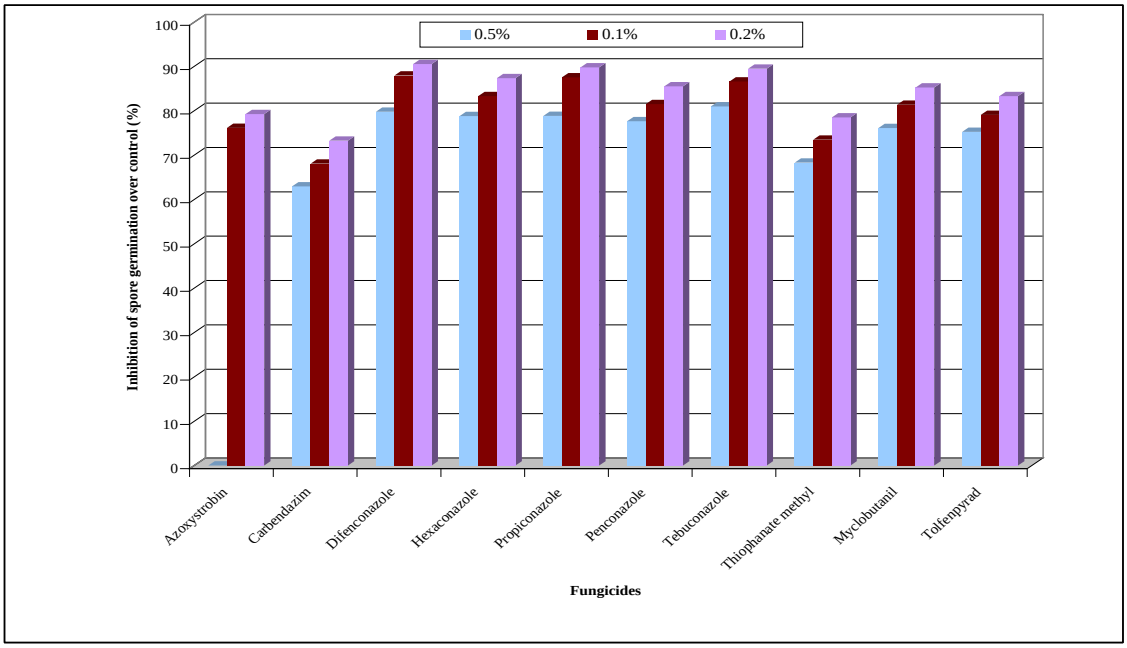

Fig. 2. *In vitro* efficacy of systemic fungicides against *Erysiphe cichoracearum* by spore germination technique.

At 0.2 per cent concentration maximum inhibition of spore germination was observed in difenconazole (90.51 %) which was on par with propiconazole (89.78 %) and tebuconazole (89.48 %) followed by hexaconazole (87.32 %) and penconazole (85.51 %). Next best treatments were myclobutanil and tolfenpyrad which recorded spore germination inhibition of 85.24 and 83.28 per cent followed by azoxystrobin (79.24 %) and thiophanate methyl (78.48 %) which were on par with each other and least inhibition (73.23 %) was observed in carbendazim.

It was noticed that in all the tested fungicides maximum inhibition was recorded in higher concentration compared to the lower concentration. However, irrespective of the concentrations of the fungicides tested, the treatment involving difenconazole (86.06 %), tebuconazole (85.67 %) and propiconazole (85.37 %) were found to be the best and significantly superior over rest of the fungicides followed by hexaconazole (83.13 %). Least mean per cent spore germination inhibition was recorded in carbendazim (68.07 %) followed by thiophanate methyl (73.41 %) and azoxystrobin (73.92 %). Similar results were noticed by Amaresh*et al*. (2013) reported that maximum mean per cent inhibition of conidial germination of *E. cichoracearum* was noticed in difenconazole (99.78 %), penconazole (98.81 %), propiconazole (98.36 %) and triadimefon (97.94 %). Bheemaraya*et al.* (2015) assessed the efficacy of seven systemic fungicides against inhibition of spore germination of *G. cichoracearum* and found that, irrespective of the fungicides nature, propiconazole across all the concentrations was found significantly superior in inhibition (94.1%) of *G. cichoracearum* which was found to be on par with penconazole (92.1 %) and hexaconazole (90.9 %). Channaveeresh and Kulkarni (2015) evaluated twelve fungicides to test the efficacy with respect to inhibition of conidial germination of *E. polygoni*. All fungicides tested were statistically significant in inhibiting the conidial germination. Azoxystrobin at 0.1 per cent concentration gave maximum per cent inhibition of conidial germination (94.16 %) followed by hexaconazole (90.51 %), myclobutanil (89.78 %) and trifloxystrobin (86.86%) respectively at 0.1 per cent concentration.

Meena *et al.* (2019) assessed the efficacy of taspa, mycobutanil, hexaconazole, karathane and propiconazole at three concentrations *viz*., 0.05 per cent, 0.1 per cent and 0.2 per cent by spore germination technique against *E. polygoni*. Results revealed that, irrespective of fungicide concentration tested, taspa recorded the maximum mean per cent inhibition of conidial germination (91.46 %) followed by hexaconazole (86.68 %) while minimum mean per cent

inhibition of conidial germination (66.55 %) was recorded in karathane. Sheethal (2020) found that the maximum mean per cent inhibition of conidial germination of *L. taurica* was observed in difenconazole (88.98 %) followed by penconazole (87.74 %) and propiconazole (86.80 %) and least inhibition was observed in carbendazim (66.42 %).

All the combi product fungicides evaluated were found effective in inhibiting spore germination of *E. cichoracearum* at three different concentrations tested. At 0.1 per cent concentration, maximum inhibition (84.35 %) of spore germination was recorded in tebuconazole 50 % + trifloxystrobin 25 % followed by hexaconazole 5 % + captan 70 % (73.23 %) and azoxystrobin 18.2 % + difenoconazole 11.4 %(72.38 %) which were found on par with each other. Next best treatments were azoxystrobin 11 % + tebuconozole 18.3 %, fenamidone 10% + mancozeb 50% and hexaconazole 4 % + zineb 68 % which recorded inhibition of 68.32, 63.23 and 61.28 per cent respectively. The least effective fungicides were copper oxy chloride 14 % + copper hydroxide 14 % with 25.48 per cent inhibition followed by carboxin 37.5 % + thiram 37.5 % and carbendazim 12 % + mancozeb 63 % with 42.32, 56.02 per cent inhibition respectively (Table 3).

Table 3: *In vitro* efficacy of combi product fungicides against *Erysiphecichoracearum* by spore germination technique

S. No.	Fungicide	Inhibition of spore germination over control (%)			
		Concentration (%)			Mean
		0.1	0.2	0.3	
1	Carbendazim 12 % + Mancozeb 63 %	56.02* (48.46)**	62.54 (52.27)	67.54 (55.27)	**62.03 (51.96)**
2	Tebuconazole 50 % + Trifloxystrobin 25 %	84.35 (66.70)	88.79 (70.49)	91.06 (72.62)	**88.06 (69.79)**
3	Fenamidone 10% + Mancozeb 50%	63.23 (52.67)	65.52 (54.04)	71.98 (58.05)	**66.91 (54.88)**
4	Hexaconazole 5 % + Captan 70 %	73.23 (58.84)	79.64 (63.18)	83.84 (66.30)	**78.90 (62.65)**
5	Hexaconazole 4 % + Zineb 68 %	61.28 (51.52)	63.64 (52.92)	72.32 (58.27)	**65.74 (54.17)**
6	Azoxystrobin 11 % + Tebuconozole 18.3 %	68.32 (55.75)	74.28 (59.53)	79.58 (63.14)	**74.06 (59.38)**
7	Carboxin 37.5 % + Thiram 37.5 %	42.32 (40.58)	52.43 (46.39)	63.36 (52.75)	**52.70 (46.55)**
8	Azoxystrobin 18.2 % + Difenoconazole 11.4 %	72.38 (58.30)	75.45 (60.30)	79.48 (63.05)	**75.77 (60.51)**
9	Copper oxy chloride 14 % + Copper hydroxide 14 %	25.48 (30.31)	37.54 (37.78)	58.45 (49.87)	**40.49 (39.52)**
Mean		**54.66 (47.67)**	**59.98 (50.76)**	**66.76 (54.79)**	
			S.Em. (±)		**C.D. at 1 %**
Fungicides (F)			0.24		0.89
Concentration (C)			0.11		0.40
F×C			0.42		1.55

*Mean of three replications, **Angular transformed value

At 0.2 per cent concentration, maximum inhibition (88.79 %) of spore germination was recorded in tebuconazole 50 % + trifloxystrobin 25 % followed by hexaconazole 5 % + captan 70 % (79.64 %). Next best treatments were azoxystrobin 18.2 % + difenoconazole 11.4 % (75.45 %) and azoxystrobin 11 % + tebuconozole 18.3 % (74.28 %) which were on par with each other. The least effective fungicides were copper oxy chloride 14 % + copper hydroxide 14 % with 37.54 per cent inhibition followed by carboxin 37.5 % + thiram 37.5 % and carbendazim 12 % + mancozeb 63 % with 52.43, 62.54 per cent inhibition respectively (Fig. 3).

At 0.3 per cent concentration, maximum inhibition (91.06 %) of spore germination was recorded in tebuconazole 50 % + trifloxystrobin 25 % followed by hexaconazole 5 % + captan 70 % (83.84 %). Next best treatments were azoxystrobin 18.2 % + difenoconazole 11.4 % (79.48 %) and azoxystrobin 11 % + tebuconozole 18.3 % (79.58 %) which were on par with each other. The least effective fungicides were copper oxy chloride 14 % + copper hydroxide 14 % with 58.45 per cent inhibition followed by carboxin 37.5 % + thiram 37.5 % and carbendazim 12 % + mancozeb 63 % with 63.36, 67.54 per cent inhibition respectively.

It was noticed that in all the tested fungicides maximum inhibition was recorded in higher concentration compared to lower concentration. However, irrespective of concentrations of fungicides tested, the treatment involving tebuconazole 50 % + trifloxystrobin 25 % recorded maximum mean per cent inhibition of spore germination (88.06 %) which is significantly superior over other treatments because tebuconazole is demethylase inhibitor interferes in process of building the structure of cell wall, finally inhibits the reproduction and further growth of fungus and trifloxystrobin interferes with respiration of plant pathogenic fungi, followed by hexaconazole 5 % + captan 70 % (78.90 %) as hexaconazole acts as antisporulant and inhibits sterol biosynthesis which is main component of fungal cell wall and captan inhibits fungal respiration and azoxystrobin 18.2 % +

difenoconazole 11.4 % (75.77 %) which inhibits mitochondrial respiration in fungi and stops their energy supply. Whereas, least mean per cent spore germination inhibition was recorded in copper oxy chloride 14 % + copper hydroxide 14 % (40.49 %) followed by carboxin 37.5 % + thiram 37.5 % (52.70 %). The results obtained in the present investigation are in close proximity with the findings of Parameshwar (2017) reported that among the various combi fungicides evaluated against *E. cichoracearum* and observed that, tebuconazole 50 % + trifloxystrobin 25 % was found to be significantly superior over other treatments and showed maximum mean per cent inhibition (87.78 %) followed by captan + hexaconazole (74.65 %) and least mean per cent inhibition was recorded in carbendazim + mancozeb (62.04 %). Mansukhbhai *et al.*, (2020) found that among the various combi product fungicides evaluated against *E. polygoni* causing powdery mildew of fenugreek, tebuconazole 50% + trifloxystrobin 25% WG recorded maximum mean spore germination inhibition (70.10 %) followed by tebuconazole 10% + sulphur 65% WG with spore germination inhibition of 66.57 per cent.

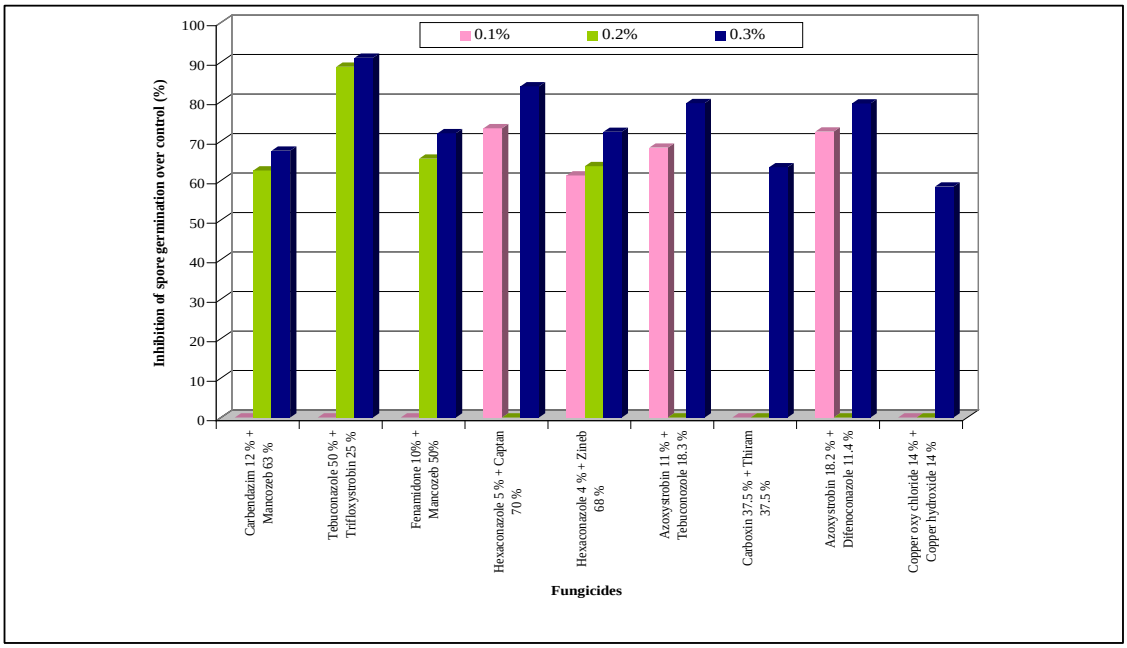

Fig. 3. *In vitro* efficacy of combi product fungicides against *Erysiphe cichoracearum* by spore germination technique.

FUTURE SCOPE

Various systemic, non-systemic and combi product fungicides which are found effective under in vitro conditions can be tested for their efficacy in controlling powdery mildew of bhendi under field conditions.

Acknowledgement. I would like to acknowledge respected chairman of my advisory committee Dr. Y. S. Amaresh, Senior scientist and Head, ICAR KVK Kawadimatti and my advisory committee members, Dr. Yenjerappa, S. T., Professor and Head, Department of Plant Pathology, College of Agriculture, UAS, Raichur, Dr. Sunil Kulkarni, Professor of Plant Pathology and Head, Agricultural Research Station, Bidar, Dr. Shekhargouda Patil, Assistant Professor, Department of Horticulture, College of Agriculture, Raichur, Dr. S. G. Hanchinal, Scientist (Entomology), AICRP on Cotton, MARS, UAS, Raichur for their fruitful and constant support, valuable suggestions and sensible criticism in animating and ameliorating this manuscript and also valuable counsel during the period of study.

Conflict of interest. Nil

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How to cite this article: Ashwini R., Amaresh, Y. S., Kulkarni, S., Yenjerappa, S. T., Patil, S. and Hanchinal, S. G. (2021). *In vitro* Evaluation of various Fungicides Against Spore Germination Inhibition of *Erysiphe cichoracearum* DC. in bhendi. *Biological Forum – An International Journal*, *13*(2): 367-373.