www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 1048-1054

www.thepharmajournal.com
Received: 14-10-2021
Accepted: 21-11-2021

Vijaykumar KN
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

Shripad Kulkarni
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

PV Patil
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

DN Kambrekar
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

TR Shashidhar
Department of Horticulture, University of Agricultural Sciences, Dharwad, Karnataka, India

Corresponding Author:
Vijaykumar KN
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

In vitro evaluation of new molecules of fungicides against purple blotch *Alternaria porri* (Ellis) Cifferi of garlic (*Allium sativum* L.)

Vijaykumar KN, Shripad Kulkarni, PV Patil, DN Kambrekar and TR Shashidhar

Abstract
Garlic (*Allium sativum* L.) is the second most widely cultivated bulb crop after onion in several countries. It is susceptible to number of diseases at various stages of plant growth. Among those, purple blotch caused by *Alternaria porri* (Ellis) Cifferi, is the most destructive disease of garlic causing yield loss about 25-60 per cent. In the present study new molecules of contact, systemic and combi product fungicides were tested against *Alternaria porri* by poison food technique at different concentrations. The contact fungicides were tested at three concentrations (0.1, 0.2 and 0.3%) and found maximum mycelial growth inhibition with mancozeb 75% WP (62.84, 74.81 and 76.42% respectively). In systemic fungicides, three concentrations (0.05, 0.1 and 0.15%) were tested and propiconazole 25% EC recorded 100 per cent inhibition at all the concentrations. The cent per cent inhibition was also noticed in tebuconazole 250 EC only at 0.15 per cent concentration. Among the combi product fungicides tested at three concentrations (0.05, 0.1 and 0.2%), maximum inhibition of mycelial growth (100%) was noticed in fluopyram 17.7% + tebuconazole 17.7% (Luna Experience 400 SC) at three concentrations and found to be most effective and significantly superior over rest of the combi product fungicides. Whereas, least mycelial growth inhibition of *Alternaria porri* was observed with chlorothalonil 75% WP (35.80%), carbendazim 50% WP (20.23%) and (iprovalicarb 5.5% + propineb 61.25% WP) (29.91%) among the contact, systemic and combi product fungicides tested respectively.

Keywords: Poison food technique, Mancozeb, Propiconazole, Luna experience

Introduction
Garlic (*Allium sativum* L.) is the second most widely cultivated bulb crop after onion and used as a spice or condiment throughout India. The letter garlic is derived from the old English word garleac which means spear leek. The origin of garlic dates back from 5000 to 6000 years. It is native to Central Asia and North-eastern Iran. It has been utilized globally for thousands of years as both food and medicine. It is the core ingredient of the Mediterranean region and used very frequently in Asian, African and European cooking. It is considered as a force of both good and evil (Parle and Vaibhav 2007) [5]. India is the second largest garlic producing country after China with the production of 17.16 lakh tonnes from 3.22 lakh hectares area with an average productivity of 5.80 tonnes per hectare. In India, major garlic producing states are Madhya Pradesh, Gujarat, Uttar Pradesh, Rajasthan, Assam, Punjab and Maharashtra. In Karnataka, garlic is grown in an area of 6.53 thousand hectares with the production of 5.42 thousand tonnes and a productivity of 0.83 tonnes per hectare (www.indianstat.com). The garlic crop is cultivated in several countries and susceptible to number of diseases at various stages of plant growth. From different parts of the world, downy mildew, rust, purple blotch, stemphylium blight and basal rot have been observed leading to substantial yield loss. These diseases also poses harmful effects during harvesting, post harvesting, processing and marketing stages, which lower the quality and export potential of the crop that significantly causes the qualitative and quantitative economic loss (Prahlad *et al*., 2021) [6]. Purple blotch caused by *Alternaria porri* (Ellis) Ciferri, is the most destructive disease of garlic. The disease is severe in high humidity of 80-90 per cent and moderate temperature of 25-30 °C. Leaves being the only photosynthesis organ directly influences the bulb yield. Significant reduction in bulb yield (25-60%) due to drying of leaves has been observed in garlic (Bisht and Agarwal, 1993) [1]. Fungicides constitute the predominate part of the control measures used against plant pathogens. Use of newer chemicals has become more popular in recent years because of their

quick results, especially in the absence of resistant varieties. Hence, different fungicides were evaluated under *in vitro* conditions to minimize the loss caused by the pathogen.

Material and Methods

The present investigation was carried out during 2019 at the Department of Plant Pathology, College of Agriculture, Dharwad, Karnataka. Different contact, systemic and combi product fungicides were tested against *A. porri* using poisoned food technique under *in vitro* conditions. The systemic fungicides were evaluated at 0.05, 0.1 and 0.15 per cent concentrations, whereas non-systemic fungicides were evaluated at 0.1, 0.2 and 0.3 per cent concentrations and combi product fungicides were evaluated at 0.05, 0.1 and 0.2 per cent concentrations. Data analyzed with ANOVA in factorial completely randomized design using IBM SPSS statistics 21 to test for significant difference among fungicides (F), concentrations (C) and their interactions (F×C). In the study, observed significant differences at 1% level of significance (P value > 0.01) for mycelial growth inhibition at different fungicides and concentrations.

The poisoned food technique (Nene and Thapliyal, 1982) was followed to evaluate the efficacy of fungicides in inhibiting the mycelial growth of *A. porri*. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted media to obtain the required concentrations. About 20 ml of poisoned medium was poured in each sterilized Petri plates. Suitable check was maintained without addition of fungicides. Eight mm mycelial disc was taken from the periphery of the colony and placed in the centre of Petri plate and incubated at 28 ± 1 °C. Three replications were maintained for each treatment. The diameter of the colony was measured after reaching maximum growth in control plates. The per cent growth inhibition was calculated by using the formula given by Vincent (1947) [10] as follows

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

Where

I = Per cent inhibition of mycelial growth.
C = Growth of mycelium in control.
T = Growth of mycelium in treatment.

Results and Discussion

Integrated disease management practice especially includes the use of fungicides to manage the disease, in the absence of resistant cultivars, or in case of sudden epidemic outbreak. There is a necessity for evaluation of fungicides under *in vitro*, which serves as a guide for testing fungicides under field conditions. Therefore, *in vitro* screening of different contact, systemic and combi products against *A. porri* was carried out in the present study.

Four non-systemic, five systemic and ten combi product fungicides were evaluated at three concentrations in the laboratory for their efficacy against *A. porri* by following poisoned food technique as explained in "Material and Methods" and data are presented in the Table 1, 2 and 3; Plate 1, 2 and 3.

All the fungicides evaluated were significantly superior over the control with respect to per cent mycelial inhibition. Among the contact fungicides tested at three concentrations (0.1, 0.2 and 0.3%), maximum per cent mycelial inhibition was recorded in treatments involving mancozeb 75% WP at all the three concentrations (62.84, 74.81 and 76.42% respectively) which was found significantly superior over rest of the treatments and it was followed by propineb 70% WP at 0.2 per cent (65.31%) and at 0.3 per cent (67.65%). The least inhibition of mycelial growth was observed in chlorothalonil 75% WP at 0.1 per cent (28.77%). Irrespective of concentrations of fungicides tested, the treatment involving mancozeb 75% WP recorded maximum mean per cent mycelial inhibition (71.36%) followed by propineb 70% WP (63.99%) and least per cent mycelial inhibition was recorded in chlorothalonil 75% WP (35.80%) (Table 1 and Plate 1).

Among the contact fungicides tested mancozeb at 0.2 per cent was found to be significantly superior showing 71.36 per cent inhibition of the mycelial growth. Similarly, (Ravichandran; Chethana *et al.* and Mishra and Gupta, 2012) [3] reported that mancozeb irrespective of concentration was highly effectively against *A. porri* under *in vitro*. Mancozeb inactivates the sulphaydryl groups of amino acids by interrupting the enzymatic activities inside the fungal cell, resulting in disruption of lipid metabolism, respiration and production of adenosine triphosphate. This might be the probable reason for inhibition in the growth of the test fungus.

Among systemic fungicides tested at three concentrations (0.05, 0.1 and 0.15%), cent per cent inhibition was noticed in propiconazole 25% EC which was significantly superior over rest of the fungicides. Tebuconazole 250 EC at 0.15 per cent concentration also showed 100 per cent inhibition. Least inhibition of 12.64, 16.39 and 31.67 per cent was noticed in carbendazim 50% WP at 0.05, 0.1 and 0.15 per cent concentration. At 0.15 per cent concentration, 96.53 and 91.81 per cent inhibition was recorded in difenoconazole 25% EC and hexaconazole 5% EC respectively and differed significantly. Irrespective of concentrations, propiconazole 25% EC recorded cent per cent inhibition of mycelial growth and least was in carbendazim 50% WP (20.23%) (Table 2 and Plate 2).

Among the tested systemic fungicides propiconazole and difenconazole recorded the maximum inhibition of mycelial growth (100%). The obtained results were in accordance with the findings made by (Wanggikar, 2012; Ravichandran, 2012 and Priyanka *et al*., 2017) [11, 7], who reported 100 per cent inhibition of mycelial growth of *A. porri*. Triazoles are the potent group of fungicides having a strong ergosterol synthesis inhibitory action which blocks the cytochrome P-450 dependant enzyme, C-14 alpha de-methylase, needed to convert lanosterol to ergosterol.

Among combi product fungicides (Table 3 and Plate 3) at all the three concentrations, maximum inhibition of mycelial growth (100%) was noticed in fluopyram 17.7% + tebuconazole 17.7% SC was found to be most effective and significantly superior over rest of the combi product fungicides. However, zineb 68% + hexaconazole 4% WG and hexaconazole 5% + captan 70% WP also inhibited 90.00 and 87.65 per cent mycelial growth at 0.2 per cent concentrations. Least inhibition was noticed in metalaxyl 64% + mancozeb 4% WP at all the three concentrations followed by carbendazim 12% + mancozeb 63% WP. Irrespective of fungicide concentration fluopyram 17.7% + tebuconazole 17.7% SC and zineb 68% + hexaconazole 4% WG were found best in inhibiting mycelial growth of *A. porri*.

Among the different combi product fungicides evaluated (fluopyram 17.7% + tebuconazole 17.7%) was found to be superior in inhibiting the mycelial growth of the pathogen compared to rest of the treatments (100%). The results

obtained where similar to the results of Yadav *et al*. (2017) [12] and Rohan *et al*. (2018) [9] who reported that fluopyram 17.7% + tebuconazole 17.7% was effective in inhibiting the growth of pathogen. Tebuconazole is a strong dimethyl inhibitor which interfere with the process building the structure of fungal cell wall thereby inhibiting the fungal germination. While, fluopyram breaks the respiratory chain in the mitochondria of the fungus cell there by blocking its energy production thus acting as a succinate dehydrogenase inhibitor (SDHI).

Table 1: Efficacy of non-systemic fungicides on inhibition of mycelial growth of *Alternaria porri*

Sl. No.	Fungicides	Per cent mycelial inhibition			Mean
		Concentrations (%)			
		0.1	0.2	0.3	
1	Captan 50% WP (Captaf)	54.44 (47.55)*	58.15 (49.69)*	64.69 (53.54)*	59.09 (50.24)*
2	Chlorothalonil 75% WP (Kavach)	28.77 (32.43)	37.78 (37.93)	40.86 (39.74)	35.80 (36.75)
3	Mancozeb 75% WP (Indofil M-45)	62.84 (52.44)	74.81 (59.88)	76.42 (60.95)	71.36 (57.64)
4	Propineb 70% WP (Antracol)	59.01 (50.19)	65.31 (53.91)	67.65 (55.34)	63.99 (53.13)
Mean		51.27 (45.73)	59.01 (50.19)	62.41 (52.18)	57.56 (49.35)
			S.Em. ±		C.D. at 1%
Fungicides (F)			0.225		0.894
Concentrations (C)			0.195		0.775
F × C			0.390		1.549

*Angular transformed values

Table 2: Efficacy of systemic fungicides on inhibition of mycelial growth of *Alternaria porri*

Sl. No.	Fungicides	Per cent mycelial inhibition			Mean
		Concentrations (%)			
		0.05	0.1	0.15	
1	Carbendazim 50% WP (Bavistin)	12.64 (20.82)*	16.39 (23.88)*	31.67 (34.24)*	20.23 (26.73)*
2	Difenoconazole 25% EC (Score)	91.81 (73.37)	93.47 (75.20)	96.53 (79.26)	93.94 (75.74)
3	Hexaconazole 5% EC (Contaf)	80.42 (63.73)	84.72 (66.99)	91.81 (73.37)	85.65 (67.74)
4	Propiconazole 25% EC (Tilt)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Tebuconazole 250 EC (Folicur)	87.50 (69.30)	91.67 (73.22)	100.00 (90.00)	93.06 (74.72)
Mean		74.47 (59.65)	77.25 (61.51)	84.00 (66.42)	78.57 (62.43)
			S.Em. ±		C.D. at 1%
Fungicides (F)			0.302		1.200
Concentrations (C)			0.262		1.039
F × C			0.523		2.078

*Angular transformed values

Table 3: Efficacy of combi product fungicides on inhibition of mycelial growth of *Alternaria porri*

Sl. No.	Fungicides	Per cent mycelial inhibition			Mean
		Concentrations (%)			
		0.05	0.1	0.2	
1	Zineb 68% + Hexaconazole 4% (Avtar 72% WG)	87.53 (69.32)*	88.77 (70.42)*	90.00 (71.57)*	88.77 (70.42)*
2	Metiram 55% + Pyraclostrobin 5% (Cabrio-Top 60% WG)	79.01 (62.73)	80.86 (64.06)	81.48 (64.51)	80.45 (63.76)
3	Fluopyram 17.7% + Tebuconazole 17.7% (Luna Experience 400 SC)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Iprovalicarb 5.5% + Propineb 61.25% (Melody Duo 66.75 WP)	7.04 (15.38)	13.95 (21.93)	41.73 (40.24)	20.91 (27.21)
5	(Tricyclazole 18% + Mancozeb 62%) (Merger 80% WP)	54.32 (47.48)	62.35 (52.15)	65.68 (54.14)	60.78 (51.23)
6	Tebuconazole 50% + Trifloxystrobin 25% (Nativo 75% WG)	74.81 (59.88)	77.28 (61.54)	86.42 (68.38)	79.51 (63.08)
7	Metalaxyl 64% + Mancozeb 4% (Ridomil Gold 68% WP)	35.12 (36.35)	34.32 (35.86)	43.33 (41.17)	37.59 (37.82)
8	Carbendazim 12% + Mancozeb 63% (Saaf 75% WP)	35.06	40.49	42.59	39.38

		(36.31)	(39.52)	(40.74)	(38.87)
9	Fenamidon 10% + Mancozeb 50% (Sectin 60% WP)	71.73 (57.88)	71.11 (57.49)	74.57 (59.71)	72.47 (58.35)
10	Hexaconazole 5% + Captan 70% (Taqat 75% WP)	84.44 (66.77)	86.79 (68.69)	87.65 (69.43)	86.30 (68.27)
Mean		62.91 (52.48)	65.59 (54.09)	71.35 (57.64)	66.62 (54.70)
			S.Em. ±		C.D. at 1%
Fungicides (F)			0.215		0.809
Concentrations (C)			0.118		0.443
F × C			0.372		1.401

*Angular transformed values

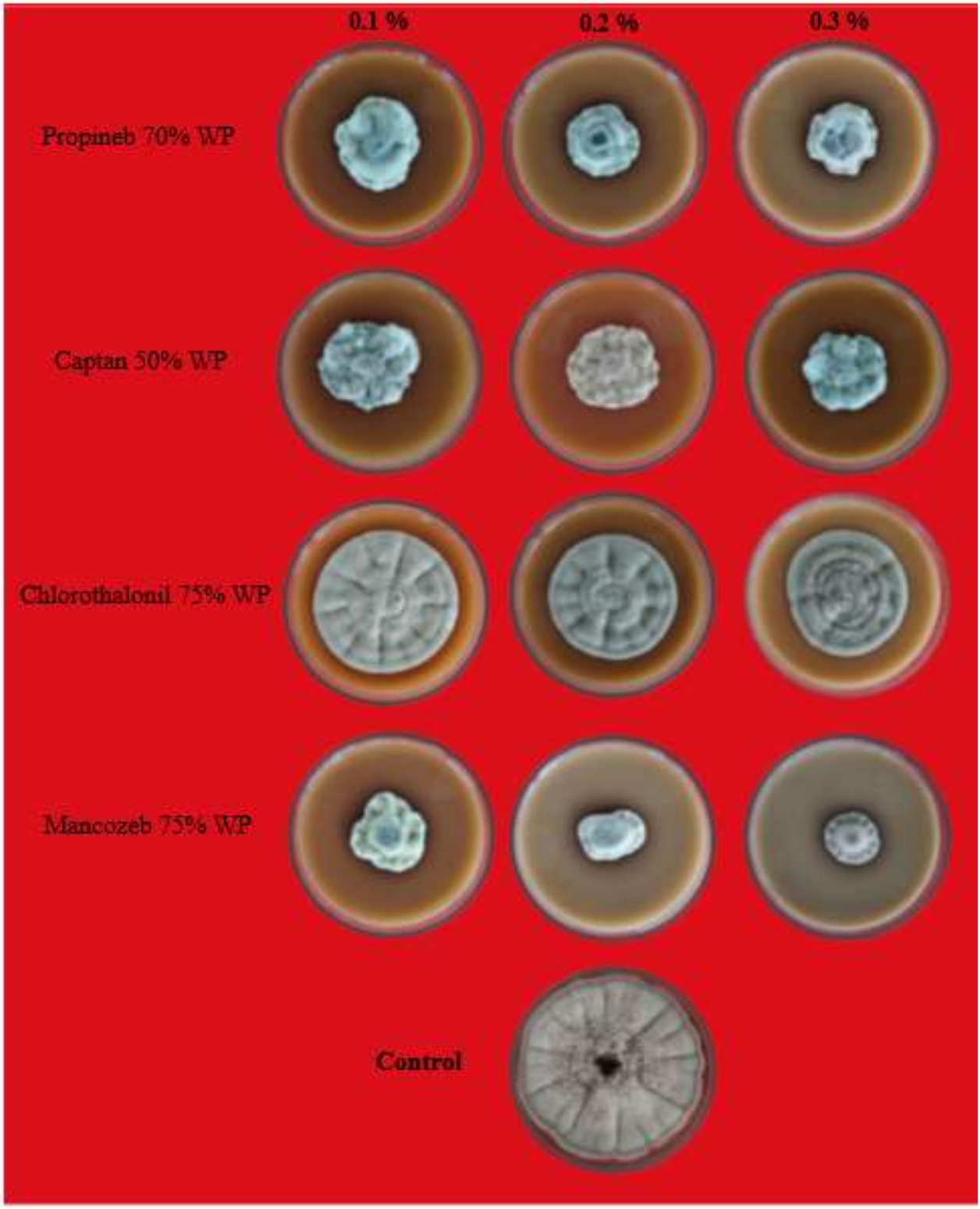

Plate 1: *In vitro* evaluation of contact fungicides against *Alternaria porri*

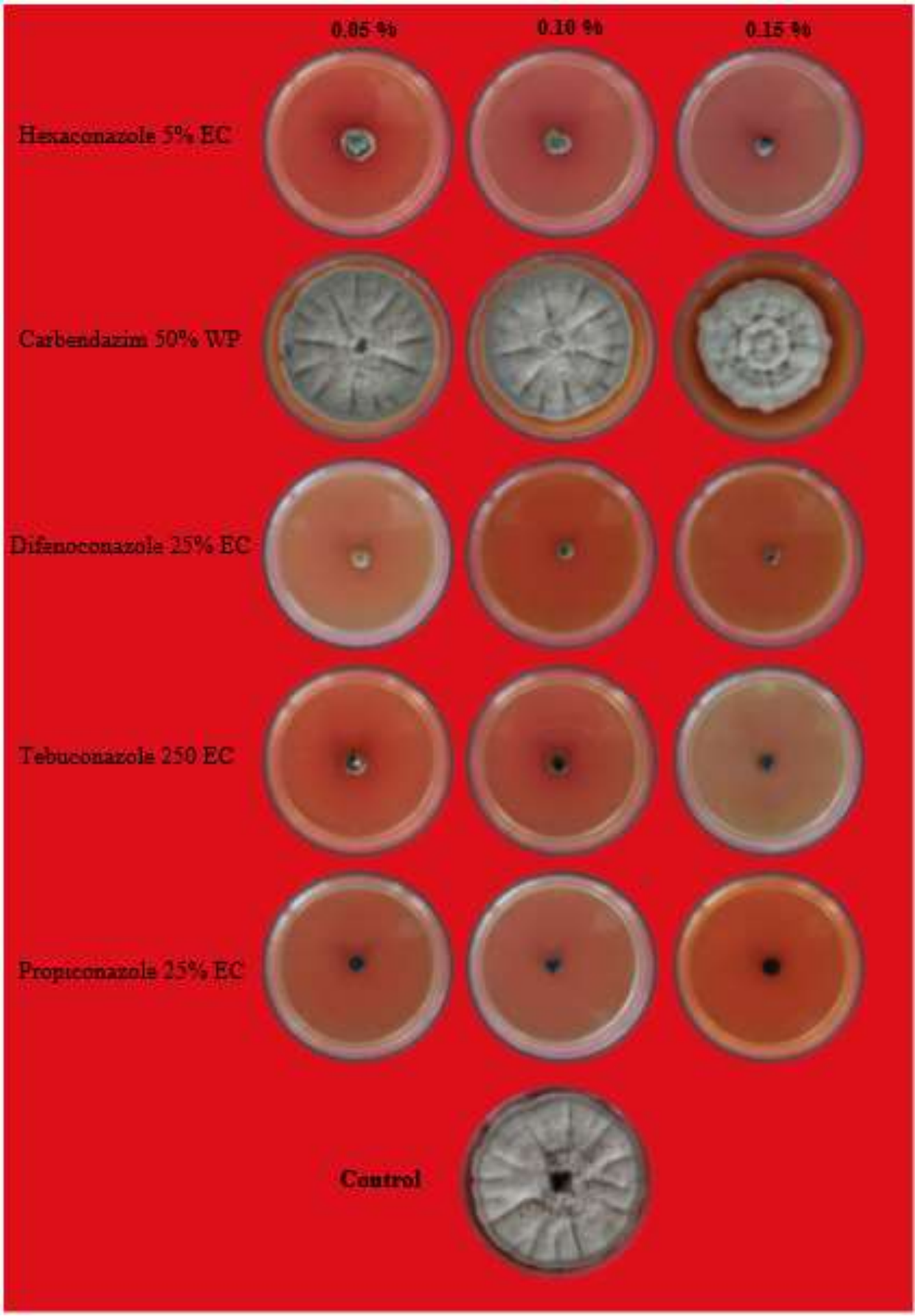

Plate 2: *In vitro* evaluation of systemic fungicides against *Alternaria porri*

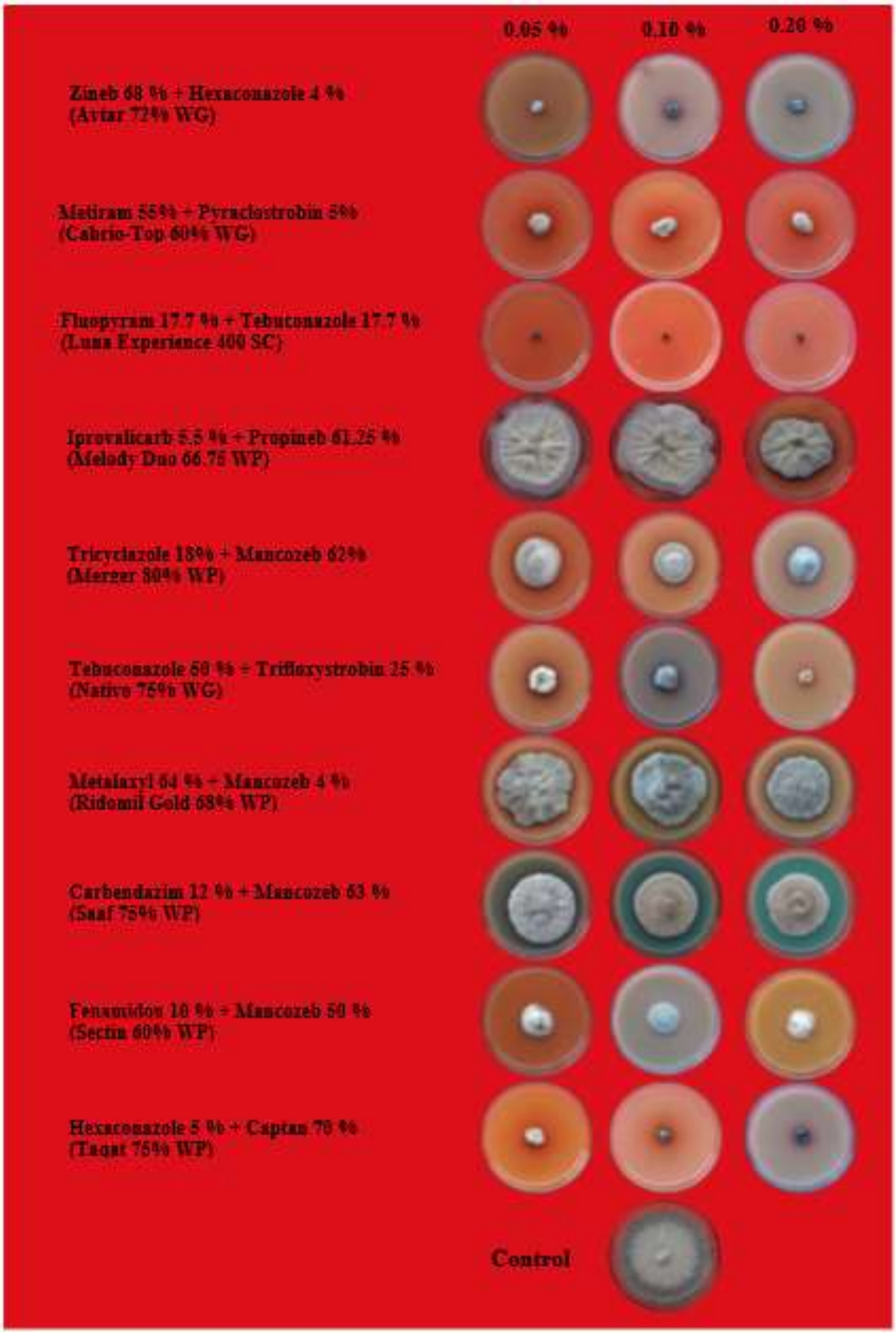

Plate 3: *In vitro* evaluation of combi product fungicides against *Alternaria porri*

Conclusion

Among the contact fungicides, irrespective of concentrations of fungicides tested, the treatment involving mancozeb 75% WP recorded maximum mean per cent mycelial inhibition (71.36%) followed by propineb 70% WP (63.99%) and least per cent mycelial inhibition was recorded in chlorothalonil 75% WP (35.80%). Among the systemic fungicides tested, propiconazole recorded 100 per cent inhibition of mycelial growth at all the tested concentrations. Least inhibition of 20.23 per cent was recorded in carbendazim 50% WP. Irrespective of fungicide concentrations among the combi product fungicides fluopyram 17.7% + tebuconazole 17.7%

SC was found as the best and inhibited cent per cent mycelial growth of *A. porri*. The least inhibition was recorded in iprovalicarb 5.5% + propineb 61.25% WP. In all the fungicides, increase in the concentration increased the inhibition of mycelial growth.

References

1. Bisht IS, Agarwal RC. Susceptibility to purple blotch (*Alternaria porri*) in garlic. Ann. App. Biol 1993;122:31-38.
2. Chethana BS, Ganeshan G, Rao AS, Bellishree K. *In vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria porri* (Ellis) Cif. causing purple blotch disease of onion. Pest manag. Hortic. Ecosyst 2012;18(2):194-198.
3. Mishra RK, Gupta RP. *In vitro* evaluation of plant extracts, bioagents and fungicides against Purple blotch and Stemphylium blight of onion. J Med. Plants Res., 2012;6(45):5658-5661.
4. Nene YL, Thapliyal PN. Fungicides in Plant Disease Control. 3rd edition, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi 1979, 325.
5. Parle M, Vaibhav K. Garlic-A Delicious Medicinal Nutrient. In: Trivedi P. C., editor, Indian Folk Medicine Jaipur. India: Pointer Publisher 2007, 210-229.
6. Prahlad, Anupriya, Godara SL, Kumhar DR, Chawla N. Prevalence and management of major diseases of garlic is it global level: A review. Agric. Rev 2021;42(1):1-8.
7. Priyanka J, Mali BL, Meena MK. Effective management of purple blotch of onion caused by *Alternaria porri* (Ellis) through host resistance, fungicides and botanicals. Int. J Curr. Microbiol. App. Sci., 2017;6(5):1737-1745.
8. Ravichandran S. Epidemiology and management of purple blotch of onion caused by *Alternaria porri* (Ellis) Cif. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India) 2012.
9. Rohan HN, Mesta RK, Kirankumar KC, Krinshna K, Jakatimath S, Pradnyarani N. *In vitro* evaluation of fungicides against purple blotch of onion caused by Alternaria porri. Int. J. Chem. Stud 2018;6(2):2489-2491.
10. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 1947;159:850.
11. Wanggikar AA. Studies on purple blotch of onion incited by *Alternaria porri* (Ellis) Cif. *M. Sc. (Agri.) Thesis*, Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra (India) 2012.
12. Yadav RK, Singh A, Jain S, Dhatt AS. Management of purple blotch complex of onion in Indian Punjab. Int. J. App. Sci. Biotechnol 2017;5(4):454-465.
13. [Weblink: https://www.indiastat.com]. [Visited on 15 September, 2019]