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Evaluation of systemic, non-systemic and combi-product fungicides against *Colletotrichum lindemuthianum* causing French Bean anthracnose

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Abstract
Anthracnose of French bean is a serious disease that restrict the production of bean around the world. It is a seed and soil borne disease. For developing its effective management strategy seven systemic fungicides, four non-systemic and three combi-product fungicides were evaluated for their efficacy against *C. lindemuthianum in vitro.* Among all the systemic fungicides tested, Tebuconazole 25.9 % EC, Propiconazole 25 % EC, Iprobenfos 48 % EC and Carbendazim 50 % WP showed cent percent mycelial inhibition at 500, 1000 and 1500 ppm and Difenoconazole 25 % EC at 1000 and 1500 ppm. Among the non-systemic and combi- product fungicides tested, Carbendazim 12% + Mancozeb 63% WP and Mancozeb 50% + Carbendazim 25% WS recorded with cent percent mycelial inhibition at 1000, 2000 and 3000 ppm, respectively.

Keywords: French bean, *colletotrichum*, fungicides, anthracnose

Introduction
French bean also knowns as 'Grain of Hope', 'Meat of the Poor' and 'Superfood' belongs to Fabaceae family (Raghupathi *et al*., 2020). It is a day neutral, shallow rooted, short duration crop. The ideal temperature for proper French bean growth is 10- 27 ℃ and below 5℃ the flowers, branches and developing pods are damaged and above 30 ℃ flowers drop is a major problem. The crop is sensitive to both water stress and water excess conditions. (Anon., 2014) [2]. In India, during 2019-2020, area of bean cultivation was 221 thousand ha. with production of 2226 thousand MT (Anon., 2021) [4]. The area and production of beans in Maharashtra during 2017- 2018 was 5.50 thousand ha. with annual production of 55.48 thousand MT (Anon., 2018) [3]. Anthracnose of French bean is a destructive disease that restrict the production of bean around the world (Kelly and Vallejo, 2004) [12]. Anthracnose is a seed and soil borne disease (Singh, 2018) and being a seed-borne disease can easily spread since farmers depend heavily on farm-saved seed and seed exchange is prevalent (Lopes and Berger, 2001) [13].

The optimum growth of *C. lindemuthianum* lies between 22°C and 23°C and maximum growth lies between 30°C and 31°C, respectively. The fungus is sensitive to high temperature but is capable of tolerating as low as -15°C to -20°C. For sporulation, it required optimum temperature of 15°C with maximum and minimum at 38°C and 4°C, respectively. Germination of the spores occurs more rapidly at temperature higher than the optimum temperature for the growth, but normally not above 27.5°C, the critical temperature ranges between 32°C and 35°C, respectively (Ravi *et al*., 2000) [17].

Colletotrichum species are reported to cause anthracnose disease in more than 121 plant genera from 45 different families, including Angiosperms, Gymnosperms, Ornamentals, Vegetables, Fruits plants, Field crops and even Grasses (Farr *et al*., 2016) [9].

Material and Methods
Isolation of pathogen
French bean showing typical anthracnose symptoms were washed, blot dried and cut into small bits, keeping half healthy and disease portion intact. The cut sample bits were further surface sterilised with 1 % NaOCl solution for 1 min followed by three sequential wash with distilled water to remove traces of NaOCl, blot dried and inoculated by keeping two bits on cooled sterilised PDA under laminar airflow cabinet. The inoculated plates were incubated in BOD at 27± 1℃ for a week. Sub-culture was done using hyphal tip technique and transferred

on PDA media under aseptic conditions and incubated at 27±1 ℃ to maintain pure culture plate.

Poison food technique

Fungicides were evaluated at three different concentrations against *C. lindemuthianum* under *in vitro* conditions using poison food technique (Nene and Thapliyal, 1993) [14]. The systemic fungicides were evaluated at 500, 1000 and 1500 ppm. Non-systemic and combi-product fungicides were evaluated at 1000, 2000 and 3000 ppm. The required quantity of fungicides was estimated, combined separately with 100 ml of sterilised PDA media in conical flask (250 ml). 20 ml of poisoned media was poured in 90 mm sterilised Petri plate and untreated control was maintained with plain PDA media. Three replications were maintained for each treatment 5 mm of seven-day old culture was seeded at each Petri plate and incubated at 27± 1 ℃. Record was done when control plate touches the periphery. The colony diameter and the per cent mycelial inhibition of the fungus was calculated using Vincent (1927) [21].

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Growth of the test fungus in control plate.
T = Growth of test fungus in treated plate.

Table 1: List of systemic fungicides used

Sl. No	Common name	Manufacturing company	Trade name
1	Azoxystrobin 25% EC	Syngenta India Ltd., Mumbai	Amitsar
2	Hexaconazole 5% EC	Greencrop Internt. Pvt. Ltd., Mumbai	Contaf -5E
3	Difenoconazole 25% EC	Syngenta India Ltd., Mumbai	Score
4	Tebuconazole 25.9% EC	Bayer crop science Ltd., Mumbai	Folicur
5	Propiconazole 25% EC	Syngenta India Ltd., Mumbai	Tilt
6	Iprobenfos 48% EC	PI Industries Ltd., Gurgaon	Kitazin
7	Carbendazim 50% WP	BASF India Ltd., Mumbai	Bavistin

Table 2: List of non-systemic and combi-product fungicides used

Sl. No	Common name	Manufacturing company	Trade name
1	Copper oxychloride 50% WP	Syngenta India Ltd., Mumbai	Blue copper
2	Propineb 70% WP	Bayer crop science Ltd., Mumbai	Antracol
3	Chlorothalonil 75%WP	Syngenta India Ltd., Mumbai	Kavach
4	Mancozeb 75% WP	Indofil Industries Ltd., Mumbai	Indofil M-45
5	Carbendazim 12% ++ Mancozeb 63% WP	United Phosphorous Ltd., Gujarat	SAAF
6	Pyraclostrobin 13.3% ++ Epoxiconazole 5% SE	BASF India Ltd., Mumbai	Opera
7	Mancozeb 50% ++ Carbendazim 25% WS	Indofil Industries Ltd., Mumbai	Sprint

Statistical Analysis

Completely Randomised Design (CRD) was used for laboratory experiment and statistical analysis was done using OPSTAT software for the present investigation.

Results and Discussion

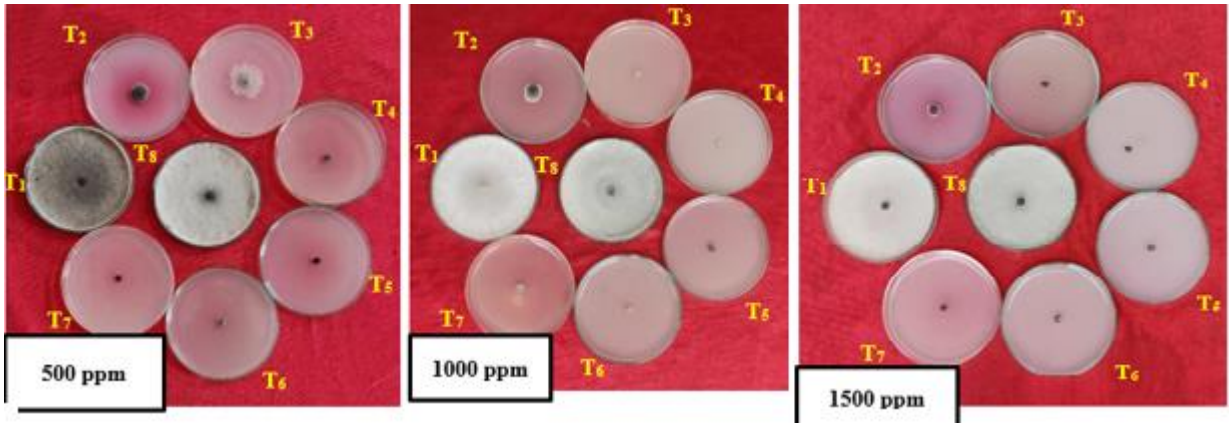
Evaluation of systemic fungicides

Result among the systemic fungicides tested at 500 ppm concentration, four fungicides *viz.*, Tebuconazole, Propiconazole, Iprobenfos and Carbendazim showed cent per cent mycelial inhibition and Difenoconazole at 1000 and 1500 ppm. This was followed by Hexaconazole 5 % EC with mycelial inhibition of 74.81 %, 80.92 % and 84.62 % at 500, 1000 and 1500 ppm respectively. Difenoconazole 25 % EC was recorded 64.06 % mycelial inhibition at 500 ppm concentration & cent per cent at 1000 and 1500 ppm. No effect of mycelial inhibition was recorded in Azoxystrobin 25 % EC (0.00 %) at all the three concentrations, respectively. (Plate 1, Table 1 and Fig 1).

Evaluation of non-systemic and combi-products fungicides

Among combi-product fungicides tested; two fungicides, Carbendazim 12% + Mancozeb 63% WP and Mancozeb 50% + Carbendazim 25% WS recorded complete mycelial growth inhibition (100.00 %) at all the three concentrations followed by Pyraclostrobin 13.3% + Epoxiconazole 5% SE with mycelial inhibition of 82.03, 86.66 and 89.62 per cent respectively. Among non-systemic fungicides, Mancozeb was found to be most effective with 50.36, 57.03 and 72.38 per cent followed by Propineb (49.25 %, 55.55 % and 62.95 %) and Chlorothalonil (12.77 %, 40.36 % and 54.44 %) against *C. lindemuthianum* at all three concentrations tested. No inhibition of mycelial growth was recorded in copper oxychloride (0.00 %) at all the three concentrations, respectively. (Plate 2, Table 2 and Fig 2).

The present results of systemic fungicides are in agreement with results obtained by Vani and Somashekhara (2018) [20] who reported cent per cent mycelial inhibition of *Colletotrichum capsica* in Difenoconazole, Propiconazole and Tebuconazole at 1000 ppm. At 500 ppm, Propiconazole inhibited cent per cent mycelial inhibition followed by Difenoconazole (97.92 %). 250 ppm, Propiconazole inhibited cent per cent followed by Difenoconazole (87.9 %). Aggarwal *et al*. (2015) [1] reported that the Tebuconazole was found to be superior with 100 per cent mycelial inhibition of *Colletotrichum lindemuthianum* at 250, 500, 1000 and 2000 ppm concentrations respectively. Jayalakshmi *et al*. (2018) [11] reported the effectiveness of Iprobenfos with mycelial inhibition of (87.99 %) at 0.15 per cent against *Colletotrichum gloeosporioides.* Carbendazim was recorded.



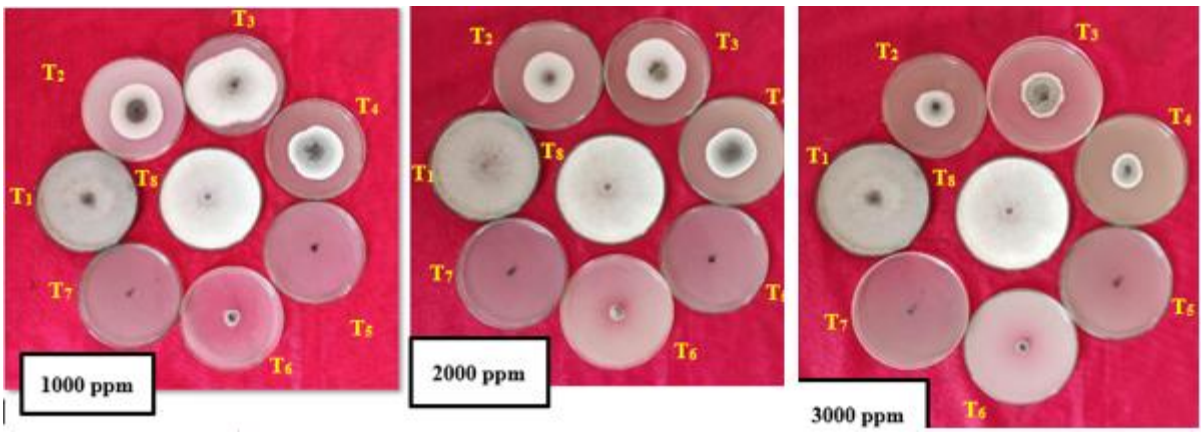
T_1	Azoxystrobin 25% EC	T_5	Propiconazole 25% EC
T_2	Hexaconazole 5% EC	T_6	Iprobenfos 48% EC
T_3	Difenconazole 25% EC	T_7	Carbendazim 50% WP
T_4	Tebuconazole 25.9% EC	T_8	Control

Plate 1: *In vitro* efficacy of systemic fungicides against *C. lindemuthianum*

Table 1: *In vitro* evaluation of systemic fungicides on *C. lindemuthianum*

Tr. No.	Treatments	Mean Colony Diameter* (mm)			% Inhibition of mycelial growth		
		500 ppm	1000 ppm	1500 ppm	500 ppm	1000 ppm	1500 ppm
T_1	Azoxystrobin 25% EC	90.00	90.00	90.00	0.00 (0.00) **	0.00 (0.00)	0.00 (0.00)
T_2	Hexaconazole 5% EC	22.66	17.16	13.83	74.81 (59.85)	80.92 (64.09)	84.62 (66.92)
T_3	Difenoconazole 25% EC	32.33	0.00	0.00	64.06 (53.15)	100.00 (90.00)	100.00 (90.00)
T_4	Tebuconazole 25.9% EC	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_5	Propiconazole 25% EC	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_6	Iprobenfos 48%EC	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_7	Carbendazim 50%WP	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_8	Control	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E. ±		0.40	0.41	0.41	0.28	0.33	0.36
C.D at 1%		1.22	1.24	1.24	0.86	1.01	1.09

* Mean of three replications.
** Figures in parenthesis are angular transformed values.



T_1	Copper oxychloride 50% WP	T_5	Carbendazim 12% + Mancozeb 63% WP
T_2	Propineb 70% WP	T_6	Pyraclostrobin 13.3% + Epoxiconazole 5% SE
T_3	Chlorothalonil 75%WP	T_7	Mancozeb 50% + Carbendazim 25% WS
T_4	Mancozeb 75% WP	T_8	Control

Plate 2: *In vitro* efficacy of non-systemic and combi-product fungicides against *C. lindemuthianum*

Table 2: *In vitro* evaluation of non-systemic and combi-product fungicides on *C. lindemuthianum*

Tr. No.	Treatments	Mean Colony Diameter * (mm)			% Inhibition of mycelial growth		
		1000 ppm	2000 ppm	3000 ppm	1000 ppm	2000 ppm	3000 ppm
T_1	Copper oxychloride 50% WP	90.00	90.00	90.00	0.00 (0.00) **	0.00 (0.00)	0.00 (0.00)
T_2	Propineb 70% WP	45.66	40.00	33.33	49.25 (44.55)	55.55 (48.16)	62.95 (52.48)
T_3	Chlorothalonil 75%WP	78.50	53.66	41.00	12.77 (20.91)	40.36 (39.41)	54.44 (47.52)
T_4	Mancozeb 75% WP	44.66	38.66	24.83	50.36 (45.19)	57.03 (49.02)	72.38 (58.28)
T_5	Carbendazim 12% + Mancozeb 63% WP	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_6	Pyraclostrobin 13.3% + Epoxiconazole 5% SE	16.16	12.00	9.33	82.03 (64.89)	86.66 (68.55)	89.62 (71.18)
T_7	Mancozeb 50% + Carbendazim 25% WS	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_8	Control	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E. ±		0.40	0.75	0.40	0.32	0.49	0.29
C.D at 1%		1.23	2.28	1.23	0.98	1.48	0.89

* Mean of three replications.
** Figures in parenthesis are angular transformed values.

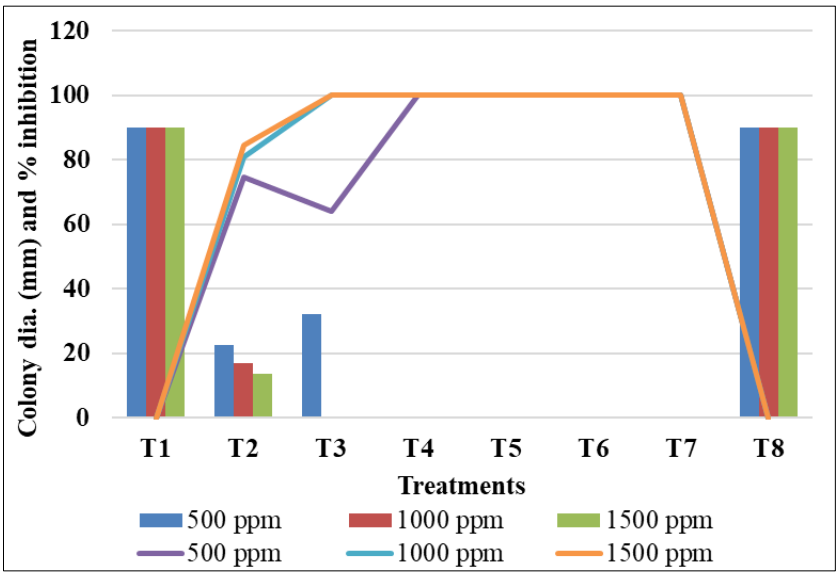


Fig 1: *In vitro* efficacy of systemic fungicides against *C. lindemuthianum*

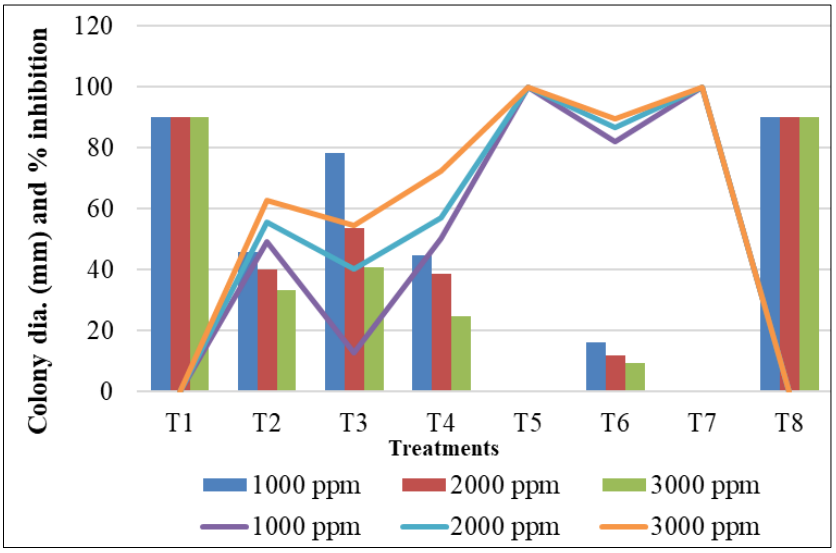


Fig 2: *In vitro* efficacy of non-systemic and combi-product fungicides against *C. lindemuthianum*

with the mycelial inhibition (90.59 %) against *Colletotrichum lindemuthianum* in the findings of Gawade *et al.* (2009) [10]. Similar results were also obtained by Badgujar *et al*. (2017) [5], Wagh *et al*. (2015) [22] and Chako and Gokulapalan (2014) [6].

Similar results of non-systemic and combi fungicides were reported by Jayalakshmi *et al*. (2018) [11], reported that Carbendazim + Mancozeb showed superior at 0.1 %, 0.2 % and 0.3 % concentration with mycelial inhibition of 68.99, 74.44 and 81.88 per cent, respectively against pomegranate anthracnose, *Colletotrichum gloeosporioides.* Similar findings were also reported by Chaudhari and Gohel (2016), Shashikumara *et al*. (2020) [18] and Poonacha *et al*. (2020) [15]. Mancozeb being effective against *Colletotrichum* was also reported by Chacko and Gokulapalan (2014) [6], Devi and Narayanaswamy (2016) [8] and Poonacha *et al*. (2020) [15]. Similar finding of Copper oxychloride with least mycelial inhibition (14.07 %) was also reported by Wagh *et al*. (2015) [22] in *Colletotrichum capsici* @ 500 ppm. Devi and Narayanaswamy (2016) [8] against *Colletotrichum lindemuthianum* @ 100 ppm with (6.32 %), 200 ppm with (8.70 %), 400 ppm with (8.95 %) and 800 ppm with (9.6 %), respectively.

Conclusion

In vitro evaluation of fungicides indicated that among seven systemic fungicides tested Tebuconazole, Propiconazole, Iprobenfos and Carbendazim showed the most effective with cent per cent mycelial inhibition at 500, 1000 and 1500 ppm and Difenoconazole at 1000 and 1500 ppm. Among non-systemic and combi-product fungicides tested, Carbendazim 12% + Mancozeb 63% WS and Mancozeb 50% + Carbendazim 25% WS was recorded the best with cent per cent mycelial inhibition.

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