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In vitro and *In vivo* evaluation of fungicides against *F. udum* f. sp. *crotalariae* in Sunhemp

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Abstract
Among the various diseases of sunhemp, fusarium wilt is causing severe loss to the sunhemp production. Managing Fusarium wilt in sunhemp is challenging because the pathogen can persist in the soil as chlamydospores for several years, even in the absence of the host plant. Consequently, fungicides have become a crucial tool in managing this disease. Assessing the efficacy of fungicides against Fusarium wilt in sunhemp is vital for developing effective disease management strategies. Hence, *In vitro* and *in vivo* evaluation of fungicides were carried out in our study. Studies on *In vitro* evaluation of fungicides revealed that, mancozeb among contact fungicides, tebuconozole among the systemic fungicides and carbendazim 12% + mancozeb 63%) WP and (carboxin 37.5% + thiram 37.5%) WP from the combi group were found effective with cent per cent inhibition of mycelial growth of the pathogen. Management of fusarium wilt of sunhemp under field condition revealed that, seed treatment with (carboxin 37.5% + thiram 37.5%) @ 3 g/kg + soil drenching of (carboxin 37.5% + thiram 37.5%) @ 0.2% or ST with tebuconozole 2% DS @ 2 g/kg + soil drenching of tebuconozole 250 EC @ 0.1% were found effective in reducing the disease incidence with maximum yield. As per B:C ratio, seed treatment with (carboxin 37.5% + thiram 37.5%) WP @ 3g/kg of seeds recorded highest B:C ratio of 2.84 and was found best.

Keywords: Fusarium wilt, efficacy, fungicide, mancozeb, tebuconozole, carboxin, thiram

1. Introduction

Sunhemp (*Crotalaria juncea*) is a versatile leguminous crop valued for its agronomic and ecological benefits. Commonly grown in tropical and subtropical areas, sunhemp serves multiple purposes, including as green manure, a cover crop, and a fiber source. It enhances soil fertility through nitrogen fixation in association with rhizobia, improves soil structure, and helps control weeds, making it a key element in sustainable agricultural practices (Singh *et al*., 2011) [24]. The crop is also noted for its quick growth and high biomass production, which further enriches soil organic matter and fertility. Additionally, sunhemp is utilized in crop rotation and intercropping to disrupt pest and disease cycles, thereby boosting overall farm productivity (Kumar *et al*., 2015) [12]. However, sunhemp cultivation is significantly hindered by Fusarium wilt, a serious disease caused by the soil-borne fungus *Fusarium oxysporum f. sp. crotalariae*. This disease is particularly destructive in sunhemp, as the pathogen enters through the roots, spreads through the plant's vascular system, and disrupts the flow of water and nutrients, leading to symptoms such as leaf yellowing, wilting, and eventually, the plant's premature death (Chakrabarti *et al*., 2014) [3]. In severe cases, the disease can cause complete crop loss, with infected plants displaying dark brown streaks within the stem's vascular tissues (Pandey & Upadhyay, 2017) [15]. Managing Fusarium wilt in sunhemp is challenging because the pathogen can persist in the soil as chlamydospores for several years, even in the absence of the host plant. This long-term survival in soil and plant debris makes crop rotation and other cultural methods only partially effective (Raghuwanshi & Gupta, 2016) [19]. Consequently, fungicides have become a crucial tool in managing this disease. However, the effectiveness of fungicides can depend on various factors, such as application timing, environmental conditions, and the specific mode of action of the fungicide (Gupta & Sharma, 2017) [9]. Assessing the efficacy of fungicides against Fusarium wilt in sunhemp is vital for developing effective disease management strategies.

Studies have shown that certain fungicides can greatly reduce disease severity and enhance the yield and quality of sunhemp crops. Systemic fungicides that inhibit fungal growth within plant tissues are particularly effective (Patil *et al*., 2018) [17]. Additionally, evaluating fungicides is essential to prevent the development of resistance in fungal populations, which could otherwise render chemical control measures ineffective over time (Bisht *et al*., 2019) [1].

The significance of fungicide evaluation extends beyond immediate disease control. By identifying the most effective fungicides and optimizing their use, farmers can reduce chemical inputs, thereby minimizing environmental impact and lowering production costs (Choudhary & Kaushal, 2020) [6]. Sustainable management of Fusarium wilt in sunhemp can be achieved by integrating fungicide use with other practices such as crop rotation, resistant varieties, and proper field sanitation (Mandal *et al*., 2021) [13].

2. Materials and Methods

2.1 Non-systemic, Systemic and Combi product fungicides used for the *In vitro* evaluation against *Fusarium udum* f. sp. *crotalaria*

The efficacy of non-systemic fungicides (0.1, 0.2 and 0.3 per cent concentration) and systemic (0.025, 0.05 and 0.1 per cent concentration) and combi product fungicides (0.1, 0.2 and 0.3 per cent concentration) against *Fusarium udum* f. sp. *crotalariae* were assayed under *In vitro* using poisoned food technique (Sharvelle, 1961) [23]. The fungicides used were mentioned below.

Table 1: Non-systemic fungicides

Sl. No.	Chemical name	Trade name	Per cent concentration
1	Captan 50% WP	Captaf	0.1%, 0.2% and 0.3%
2	Mancozeb 75% WP	Indofil M-45	
3	Copper oxychloride 50% WP	Blitox	
4	Chlorothalonil 75% WP	Kavach	
5	Propineb 65% WP	Anthracol	

Table 2: Systemic fungicides

Sl. No.	Chemical name	Trade name	Per cent concentration
1	Thiophanate methyl 70% WP	Roko	0.025%, 0.05% and 0.1%
2	Propiconazole 25% EC	Tilt	
3	Hexaconazole 5% EC	Contaf	
4	Carbendazim 50% WP	Bavistin	
5	Difenconazole 25% EC	Score	
6	Tebuconazole 250 EC	Folicur	

Table 3: Combi product fungicides

Sl. No.	Chemical name	Trade name	Per cent concentration
1	(Carbendazim 12%+ Mancozeb 63%) WP	SAAF	0.1%, 0.2% and 0.3%

2.1.1 Poisoned food technique

The efficacy of the fungicides was tested against the pathogen by using poisoned food technique. The required concentrations of chemicals was weighed and incorporated into sterilized, cooled potato dextrose agar. Twenty ml of cooled molten PDA medium was poured into 90 mm sterilized Petri dishes and all the plates was inoculated with actively growing seven mm mycelial disc of the pathogen separately. Three replications were maintained for each treatment. These plates were incubated at 27±1 °C for seven days and colony diameter was recorded. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947) [25].

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

Where, I = Per cent inhibition of mycelial growth

C = Radial growth in control (mm)

T = Radial growth in treatment (mm)

2.2 Methodology for *In vivo* evaluation of fungicides against wilt of sunhemp.

Effective chemicals under *In vitro* conditions was tested under field experiment during *rabi* in the year 2019-2020 at Main Agricultural Research Station, University of Agricultural sciences, Dharwad. Randomised Block Design was followed with 10 treatments and 3 replications. Size of the plot was 5 m × 4 m and spacing followed was 30 cm × 10 cm.

Table 4: Details of the treatments are given below

Treatments	Description
T_1	Seed treatment with most effective contact fungicide
T_2	Seed treatment with most effective systemic fungicide
T_3	Seed treatment with most effective combi product fungicide
T_4	Soil drenching with most effective contact fungicide
T_5	Soil drenching with most effective systemic fungicide
T_6	Soil drenching with most effective combi product fungicide
T_7	T_1+ T_4
T_8	T_2+T_5
T_9	T_3+ T_6
T_{10}	Untreated control

2.2.1 Observations recorded

a. Per cent disease incidence

Per cent disease incidence (PDI) was calculated by using the formula given by Wheeler (1969) [26].

$$\text{Per cent Incidence} = \frac{\text{No. of plants showing wilting symptoms}}{\text{Total number of plants observed in a field}} \times 100$$

b. Yield

At physiological maturity, sunhemp plants from respective treatments were collected and kept it for drying under sunlight. After complete drying they were threshed and winnowed. Yield per hectare was computed by using net plot yield data and it was then converted to quintal per hectare.

2.3 Statistical analysis

The statistical analysis of experiment was carried out as per the procedure given by Panse and Sukathme (1967) [16]. Per cent data was transformed in to arc sine values and were analyzed statistically. The data obtained in the present investigations for various parameters were subjected to ANOVA for a completely randomized design for *In vitro* and randomized block design for *in vivo*.

3. Results and Discussion

3.1 *In vitro* evaluation of fungicides against *Fusarium udum* f. sp. *crotalariae*

3.1.1 *In vitro* efficacy of non systemic fungicides against *Fusarium udum* f. sp. *crotalariae*

Five non systemic fungicides were tested against *F. udum* f. sp. *crotalariae* for their efficacy. Among the tested fungicides, mancozeb showed the maximum mean inhibition of mycelial growth (100%) which was significantly superior over rest of the fungicides. Copper oxychloride (69.51%) was found next best fungicide followed by propineb (40.25%) and captan (34.69%). Least mycelial inhibition was observed in chlorothalonil (31.23%).

At 0.1 per cent concentration maximum mycelial inhibition was recorded in mancozeb (100%). Next best fungicides observed were captan (30.74%) followed by chlorothalonil (19.63%) and propineb (10.00%). Copper oxychloride recorded the least mycelial inhibition of 8.52 per cent.

At 0.2 per cent concentration, mancozeb and copper oxychloride showed complete inhibition of mycelial growth followed by propineb (41.85%) and captan (33.70%). Least mycelial inhibition was observed in chlorothalonil (24.07%).

At 0.3 per cent concentration, both fungicides copper oxychloride and mancozeb recorded cent per cent mycelial inhibition followed by propineb (68.89%) and chlorothalonil (50.00%) and the least inhibition was recorded in captan (39.63%).

The results were in confirmative with the findings of Chaudhary *et al*. (2013) [4] and Shah *et al*. (2006) [21] that the mancozeb was found effective in inhibiting *Fusarium udum* by showing maximum inhibition as compared to other non systemic fungicides. Action of mancozeb fungicide is to inhibit the germination, growth and multiplication of the fungus (Nene and Thapliyal, 1973) [14].

The results were also in agreement with the findings of Kulkarni (2006) [11] that mancozeb was found best over other nonsystemic fungicides against the growth of *F. oxysporum* f. sp. *gladioli*.

3.1.2 *In vitro* efficacy of systemic fungicides against *F. udum* f. sp. *crotalariae*

Efficacy of six systemic fungicides were evaluated against *F. udum* f. sp. *crotalariae* by poisoned food technique. Tebuconozole recorded maximum mean inhibition (100%) and was found significantly superior over the rest of the fungicides tested which was followed by carbendazim recording mean mycelial inhibition of 72.22 per cent. Next best fungicides were found to be thiophanate methyl (58.40%) and propiconozole (47.78%). Least mean mycelial inhibition among the systemic fungicides was observed in difenconozole (43.21%) and hexaconozole (31.11%)**.**

At 0.025 per cent concentration, cent per cent mycelial inhibition was recorded in tebuconozole and was significantly superior over all other fungicides. Next best were found to be carbendazim (65.56%) followed by thiophanate methyl (48.15%). Least mycelial inhibition was recorded in propiconozole (40.74%), difenconozole (32.22%) followed by hexaconazole (19.26%).

At 0.05 per cent concentration, complete inhibition of mycelial growth was recorded by tebuconozole (100%). Next best fungicides recording highest mycelial inhibition was found to be carbendazim (74.07%) followed by thiophanate methyl (56.30%). Propiconozole (44.44%) and difenconozole (44.07%) were stastistically on par with each other. Hexaconozole (37.78%) recorded the least mycelial inhibition.

At 0.1 per cent concentration tebuconozole showed cent percent mycelial inhibition followed by carbendazim (77.04%) and thiophanate methyl (70.74%). Next best fungicides observed were propiconozole (58.15%) followed by difenconozole (53.33%). Least mycelial inhibition was observed in hexaconozole (46.30%) (Fig. 2)

Among different concentrations tested, it was found that the maximum mycelial inhibition (67.59%) was observed with 0.1 per cent followed by 0.05 per cent (57.78%) and 0.025 per cent (50.99%).

The results obtained are in line with the work of Chennakesavulu *et al*. (2013) [5] wherein complete mycelial inhibition was observed in tebuconazole against *Fusarium udum.* Triazole fungicide, tebuconazole was effective because it interfere with the biosynthesis of fungal sterols and act as ergosterol biosynthesis inhibitor. In most of the plant pathogenic fungi ergosterol is an important component of cell wall structure and its absence leads to damage to the cell wall and death of the fungal cell and carbendazim being a benzimidazole group that interfere with cell wall synthesis and energy metabolism of fungi as reported by Nene and Thapliyal (1973) [14]. The experimental result is in confirmative with Patil (2012) [17] that tebuconazole inhibited the growth of *F. oxysporum* f. sp. *cepae* at all three concentrations (0.025, 0.05, 0.1%) tested.

3.1.2. *In vitro* efficacy of combi product fungicides against *F. udum* f. sp. *crotalariae*

Five different combi product fungicides were tested for their efficacy at three different concentrations of 0.1, 0.2 and 0.3 per cent. Among them, (carbendazim 12% + mancozeb 63%) WP and (carboxin 37.5% + thiram 37.5%) WP recorded cent per cent mycelial inhibition in all the three different concentrations. Next best fungicide found was (trifloxystrobin 25% + tebuconazole 50%) WG recording mean mycelial ion of 81.11 per cent followed by (tricyclazole 25% + mancozeb 63%) WP with 65.43 per cent of mean mycelial inhibition. Least mean mycelial inhibition of 62.96 per cent was recorded by (captan 70% + hexaconozole 5%) WP. (Fig.3).

Similar line of observations have been recorded by different researchers by Divyabharathi and Benagi (2018) that vitavax power recorded cent per cent inhibition of *Fusarium solani* causing wilt of betel vine. The results are in agreement with the work of Ravichandran and Hegde (2015), that carbendazim 12% + mancozeb 63% (Saaf) was effective in all concentrations (0.1, 0.2 and 0.3%) with cent per cent inhibition against *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea. The combi product fungicides have combinations of contact, systemic fungicides. These groups of fungicides have mode of action of both contact as well as systemic fungicides (El-Deeb *et al*., 2002).

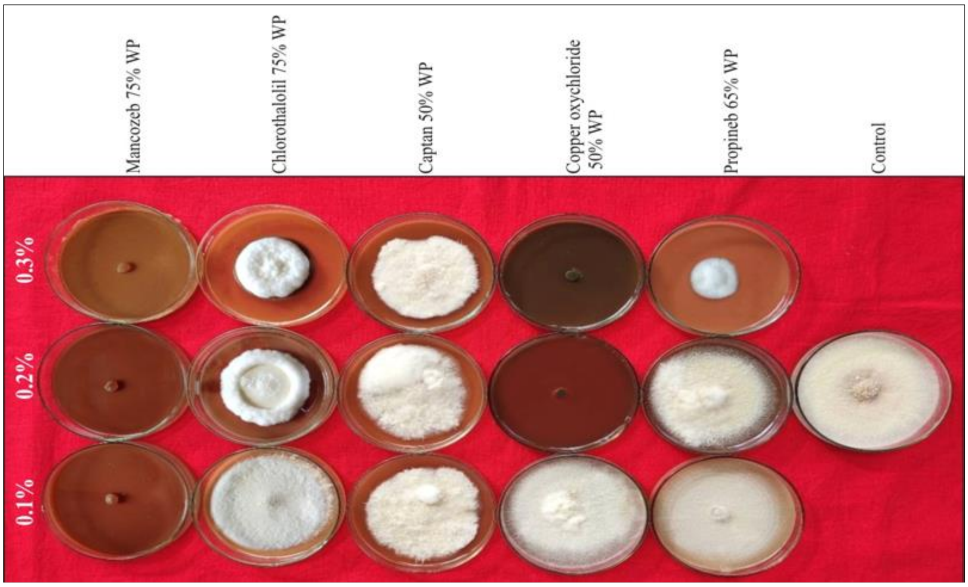

Fig 1: In vitro efficacy of non systemic fungicides against *Fusarium udum f.* sp. *Crotalariae*

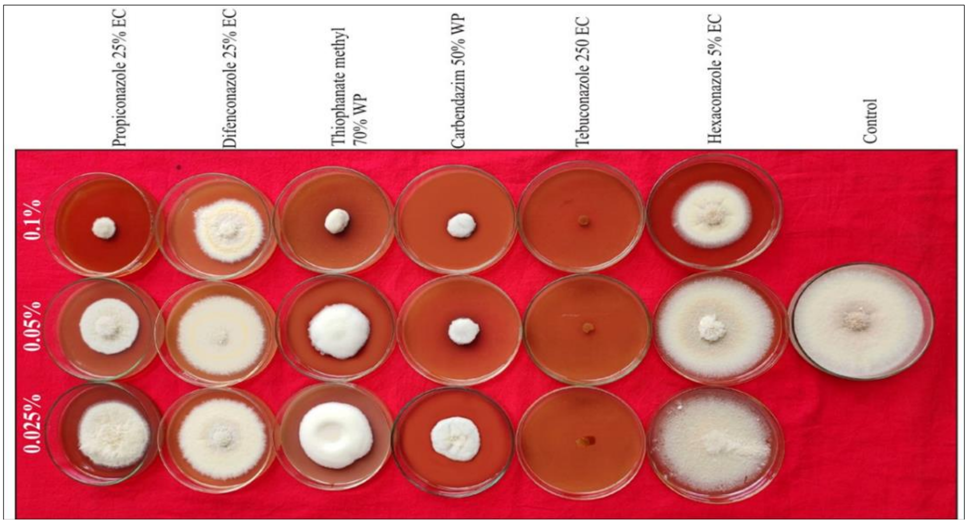

Fig 2: *In vitro* efficacy of systemic fungicides against *Fusarium udum* f. sp. *Crotalariae*

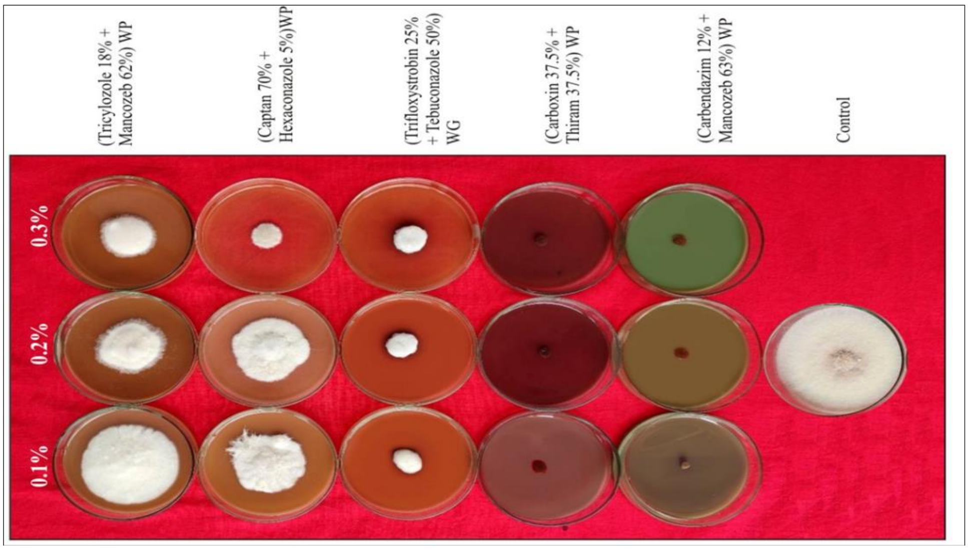

Fig 3: *In vitro* efficacy of combi product fungicides against *Fusarium udum* f. sp. *crotalariae*

3.2 *In vivo* evaluation of fungicides against *Fusarium udum* f. sp. *crotalariae*

Ten different treatments were evaluated against Fusarium wilt of sunhemp under field condition. Among the treatments tested, seed treatment with (carboxin 37.5% + thiram 37.5%) @ 3 g/kg + soil drenching of (carboxin 37.5% + thiram 37.5%) @ 0.2% recorded lowest per cent disease incidence (PDI) of 20.27 per cent with the maximum disease reduction over control of 74.75 per cent (T_9) and was statistically on par with ST with tebuconozole 2% DS @ 2 g/kg + soil drenching of tebuconozole @ 0.1% (T_8) having PDI of 22.27 per cent with per cent reduction over control of 72.26. Maximum yield of 11.13 q/ha was recorded in T_9 [(carboxin 37.5% + thiram 37.5%) @ 3 g/kg + soil drenching of (carboxin 37.5% + thiram 37.5%) @ 0.2%] whereas, control plot recorded the least yield of 3.41 q/ha and the highest B:C ratio of 2.84 was recorded in T_3.. seed treatment with (carboxin 37.5% + thiram 37.5%) @ 3 g/kg followed by treatment T_2 seed treatment with tebuconozole 2% DS @ 2 g/kg with the untreated control recording the least B:C ratio of 1.01 (Table 1)

The result is in accordance with Hegde *et al*. (2014) that the wilt complex in coleus managed effectively by dipping the cuttings in 0.1% carboxin + thiram before planting followed by drenching the same fungicide @ 0.1% and also observed that maximum yield was recorded in the same treatment.

Combi fungicide (carboxin 37.5% + thiram 37.5%) WP, has mode of action of both contact and systemic fungicides, cause suppression of pathogen by inhibiting the growth and development. Tebuconozole, a triazole fungicide have potential to manage soil borne pathogen by inhibiting the C-14 α demethylation of 24- methylene dihydrolanosterol, a precursor of ergosterol in fungi (Brent and Hollomon, 1995) [2].

Table 1: *In vivo* evaluation of fungicides against of wilt of sunhemp caused by *Fusarium udum* f. sp. *Crotalariae*

Treatment No.	Treatment details	Percent Disease Incidence	Per cent disease reduction over control	Yield (q/ha)	B:C ratio
T_1	ST with mancozeb 75% WP @ 2gm / kg of seeds	40.93 (39.62)*	49.00	7.26	2.29
T_2	ST with tebuconozole 2% DS @ 2 gm/ kg of seeds	35.60 (36.62)	55.64	8.30	2.62
T_3	ST with (carboxin 37,5% + thiram 37.5%) WP @ 3 gm/ kg of seeds	30.27 (33.36)	62.29	9.04	2.84
T_4	SD with Mancozeb 75% WP @ 0.2%.	34.27 (35.82)	57.31	8.35	1.96
T_5	SD with tebuconozole 250 EC @ 0.1%.	31.86 (34.35)	60.30	8.68	1.41
T_6	SD with (carboxin 37,5% + thiram 37.5%) WP @ 0.2%.	27.73 (31.76)	65.45	9.36	1.60
T_7	ST with mancozeb @ 2gm / kg of seeds + SD with mancozeb @ 0.2%	25.06 (30.03)	68.77	9.67	2.27
T_8	ST with tebuconozole 2% DS @ 2 gm/ kg of seeds + SD with tebuconozole 250 EC @ 0.1%	22.27 (28.14)	72.26	10.47	1.69
T_9	ST with (carboxin 37,5% + thiram 37.5%) WP @ 3 gm/ kg of seeds + SD with (carboxin 37,5% + thiram 37.5%) WP @ 0.2%	20.27 (26.74)	74.75	11.13	1.82
T_{10}	Untreated control	80.26 (63.60)	-	3.41	1.01
	SEm±	1.49		0.15	
	C.D. @ 5%	4.46		0.44	
	C.V. (%)	7.17		13.41	

*Angular transformed values ST – Seed treatment SD – Soil drenching

4. Conclusion

In the study evaluating the efficacy of fungicides against *Fusarium udum f. sp. crotalariae*, mancozeb and tebuconazole were found to be the most effective in inhibiting mycelial growth among non-systemic and systemic fungicides, respectively. Mancozeb showed complete inhibition at higher concentrations, while tebuconazole demonstrated similar results across all tested concentrations. Among combi product fungicides, carbendazim + mancozeb and carboxin + thiram provided full inhibition at all concentrations. These results align with previous studies, confirming the fungicidal potency of these products against Fusarium species through multiple mechanisms, including disruption of fungal sterol biosynthesis and cell wall integrity.

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