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Exploring the Antifungal Efficacy of Botanicals, Bioagents and Fungicides against *Ephelis japonica* Causing Udbatta Disease of Rice

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Abstract

Udbatta, also known as Black choke, is a significant seed-borne disease of rice caused by *Ephelis japonica*. The disease is sporadic and can lead to complete yield loss in affected plants. The effectiveness of systemic, non-systemic, combination fungicides, 11 botanicals and eight bioagents was evaluated against *E. japonica*. Complete inhibition (100%) of mycelial growth was achieved with the systemic fungicide Tebuconazole 45 per cent SC and the combination fungicide Carbendazim 12% + Mancozeb 63 per cent WP. The contact fungicide Copper oxychloride 50 per cent WP resulted in a mean mycelial inhibition of 62.60 per cent. Seed treatment with Carbendazim 12% + Mancozeb 63 per cent WP using the rolled towel method, was found with the lowest seed infection rate (1.00%), the highest germination rate (99.02%), the longest seedling length (20.03 cm) and the highest seedling vigor index (1965.85). Among the plant extracts tested against the test pathogen, garlic bulb extract inhibited the highest (77.18%), while *Calotropis procera* leaf extract inhibited the least (19.33%). Dual culture technique against test pathogen using bioagents revealed that *Trichoderma harzianum* and *T. hamatum* isolatesto demonstrate complete inhibition (100%), while *T. asperellum* with lower inhibition rate (74.20%). In the double Petri dish assay, *T. harzianum* (Th-41) caused the highest mycelial inhibition (77.24%), while the least inhibition was observed in *T. asperellum* (Ta-6) (54.44%).

Keywords : *Ephelis japonica*, Fungicides, Poisoned food technique, Rice, Udbatta

The most widely grown food crop in the world, rice (*Oryza sativa* L.) grows well in a variety of agroclimatic conditions from coastal plains to hilly areas. In India, rice is grown in 45.76 million hectares with an average productivity of 2,717 kg per hectare, yielding 124.36 million tons. In Karnataka, a yield of 4.34 million tons and a productivity of 3.72 tons per hectareis obtained when cultivated over 1.41 million hectares (Anonymous, 2024). Even though rice is grown on the largest area in India, its production is still quite low. Diseases are one of the many biotic factors influencing rice production and they present a major problem in many tropical and temperate rice-growing habitats. According to estimates, rice diseases often result in losses of 10 to 15 per cent per year worldwide (Alase *et al*., 2021).

Blast, brown leaf spot, bacterial blight, sheath blight, sheath rot, udbatta, root knot nematode, false smut, and tungro are the main rice diseases found in Southern Karnataka resulting in significant economic loss (Chethana *et al.,* 2016). Recently, udbatta disease caused by *Ephelis* sp. has gained destructive prominence in areas like Mandya, where it was

previously sporadic or endemic was first reported in India by Sydow in 1914 with its telomorphic stage identified as *Balansia oryzae*, a member of the Ascomycetes class.

Udbatta-infected plants remain indistinguishable until the boot leaf stage, where in infected earheads emergeas erect, grayish-white, cylindrical structures resembling incense sticks 'Agarbatti' that replace normal earheads (Christensen *et al*., 2000 and CPC, 2005). West Africa, Hong Kong, New Caledonia and Southwest China have reported the disease. The disease is prevalent in Indian states such as Bihar, Odisha, Maharashtra and Karnataka, particularly in low-hill regions with high soil temperatures (~28°C) and moisture. Reports of udbatta disease in other host plants like *Cyanodon dactylon*, *Leptochloa chinensis*, *Echinochloa crusgalli*, *Panicum* spp., *Pennisetum* spp. and *Eragrostis tenufolia* was reported (Govindu and Thirumalachar, 1961).

In endemic areas, udbatta disease can result in significant yield losses even though it is typically sporadic and of little consequence. Its prevalence in Karnataka has recently increased due to the heightened susceptibility of various rice varieties (Sannegowda and Pandurangegowda, 1986). During 2022-24, the disease severely affected popular varieties like Jyothi, Jaya, Tunga, MTU 1001 and BR2655. During kharif season of 2018, disease severity reached up to 50 per cent in the Tunga variety in the Sakleshpura area of Hassan district, while in BR2655 and MTU 1001, it ranged from 10 to 30 per cent in parts of Mandya district. In infected plants, the entire panicle was affected and even a single infected panicle resultedin complete yield loss. Seed-borne pathogens cause both pre- and post-infection problems, resulting in substantial quality losses (Neergaard, 1977).

Utilizing resistant cultivars and modifying agronomic practices are examples of cultural controls that help prevent disease development, but they may also restrict productivity. These methods are frequently time-consuming, labor-intensive and economically unviable for a large number of farmers. Fungicide application is now a typical approach to control disease. The effectiveness of several novel fungicides and combinations in treating rice disease has been demonstrated, but nothing is known about how well they work against udbatta disease. The pathogen's main source and carrier is the seed. *E. japonica* adopts to the environment when infected seeds are sown, creating infection foci that swiftly spread to nearby plants, greatly intensifying the disease (White *et al*., 1995). It is essential to treat seeds with fungicides in order to control this seed-borne disease early. The main recommendation for managing udbatta disease at the moment is hot water treatment, however farmers may not always find this feasible. Developing efficient seed treatment methods can improve disease control effectiveness and reduce the spread of udbatta disease in rice farming (Prasannakumar and Shankar., 2015).

This study focuses on evaluating fungicides, botanicals, bioagents and seed treatments with fungicides *in vitro* against rice udbatta pathogen. Notably, the assessment of fungicides, botanicals and bioagents presented here is the first of its kind for this disease. The findings provide a preliminary screening guide for fungicides effective in inhibiting *E. japonica* growth, contributing to the disease management.

Material and Methods

Isolation of the Fungus

The spore drop technique was used to isolate the causative agent of udbatta disease from rice panicles collected from Mandya that exhibited typical symptoms. Later, a portion of the fungal growth was transferred to PDA slants for culture maintenance and purification.

In vitro Evaluation of Fungicides

Using the poisoned food technique and potato dextrose agar (PDA) as the basal medium, the fungicides (contact, systemic and combination products) were assessed *in vitro* against *Ephelis japonica*, the causal agent of udbatta disease in rice. To the 250 ml conical flasks, 100 ml of sterilized PDA was mixed thoroughly to ensure an even dispersion of a defined quantity of

each fungicide. Sterilized Petri dishes were then filled with 20 milliliters of the fungicide-amended medium. Following solidification, 5 mm disc of test pathogen and bioagent that are seven days old were inoculated to each plate and the plates were then incubated for seven days at 27 ± 2°C and radial mycelial growth was measured (Vincent, 1947). The resulting data were averaged and analyzed statistically. Using the formula provided by Nene and Thapliyal (1982), the percentage suppression of the fungus's mycelial growth was computed.

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

Where, I = per cent inhibition

C = growth in control

T = growth in treatment

The treatments included,

Contact Fungicides : (Chlorothalonil 75% WP, Copperoxychloride 50% WP, Zineb 75% WP, Captan 50 % WP, Propineb 70% WP, Mancozeb 75% WP).

Systemic Fungicides : (Propiconazole 25% EC, Difenoconazole 25% EC, Carbendazim 50% WP, Hexaconozole 5% EC, Tebuconazole 25.9% EC, Azoxystrobin 25% SC) and

Combination Products : (Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Fluropyram 200g/L + Tebuconazole 200g/L SC, Fluopyram 250g/L + Trifloxystrobin 250g/L SC, Zineb 68% + Hexaconozole 4% WP, Carboxin 37.5% + Thiram 37.5% DS, Azoxystrobin 18.2% + Difenconazole 11.45% SC).

In vitro Evaluation of Seed Treatment with Fungicides in Rolled Towel Method

Seeds of the susceptible paddy variety 'Jyothi' were surface sterilized and artificially inoculated with *E. japonica* by soaking them in a fungal spore suspension for 24 hours. As a control, a separate batch of seeds were soaked in sterile distilled water. After inoculation, the seeds were air-dried and treated with three different concentrations (0.1%, 0.2% and 0.3%) of various fungicides, including Mancozeb 75% WP, Copper oxychloride 50% WP, Tebuconazole 45% SC, Carbendazim 50% WP, Carboxin 37.5% + Thiram 37.5% DS and Carbendazim 12% + Mancozeb 63% WP. The treated seeds were air-dried again and subjected to the rolled paper towel method, as described by Anonymous (1996). The seeds were placed on moistened germination paper and incubated in a germination chamber for 14 days. Data was collected for seedling vigor index, germination percentage and seed infection percentage calculation. Shoot length was measured from the stem base to the growing point of the youngest leaf.

- The seedling vigor index was calculated using the formula proposed by Abdul Baki and Anderson (1973)

  Vigour Index = (Root length + Shoot length) cm
  ×
  Per cent of seed germination

- The percentage of seed infection associated was calculated using the formula provided by Jha (1995)

$$\text{Per cent seed infection} = \frac{\text{Number of seeds infected}}{\text{Total Number of seeds}} \times 100$$

In vitro Evaluation of Bio-Control Agents

The antagonistic potential of six fungal and two bacterial bioagents against *Ephelis* sp. was evaluated *in vitro* using a dual culture technique and the efficacy of volatile organic compounds produced by fungal biocontrol agents was evaluated using double Petri dish assay (Andargie *et al*., 2017 and Vanama *et al*., 2024).

a) Dual Culture Technique

Eight antagonists- *Trichoderma harzianum* (Th-41), *T. asperellum* (Ta-6), *T. viride* (Tv-2), *T. harzianum* (Th-44), *T. hamatum* (Th-14), *Bacillus subtilis* (Bs-O), *T. viride* (Tv-M), and *Pseudomonas fluorescens* (Pf-M) were evaluated *in vitro* against *Ephelis japonica* using the dual culture method

(Dennis and Webster, 1971). Potato dextrose agar (PDA) was used as the growth medium for both the pathogen and the antagonists. Twenty milliliters of PDA were aseptically poured into each Petri dish and allowed to solidify. A five mm mycelial disc of the test fungus and an antagonist was placed on opposite sides of the PDA plates. Each treatment was replicated three times and the plates were incubated at 27 ± 2°C for seven days. Radial mycelial growth was observed for all replicated treatments and the percentage inhibition of pathogen growth was determined using the dual culture method. The data were statistically analyzed and averaged.

b. Double Petri Dish Assay

A five mm diameter mycelial disc of actively growing fungal antagonists (*T. viride* Tv-2, *T. harzianum* Th-41, *T. asperellum* Ta-6, *T. viride* Tv-M and *T. hamatum* Th-14) from a seven-day-old culture on PDA medium was placed at the center of the basal lid of a Petri dish containing sterile PDA. Another basal lid containing sterile PDA, inoculated with a five mm diameter mycelial disc of the target pathogen, replaced the upper lid of the Petri dish. The two plates were sealed together with sellotape to create airtight conditions and was incubated at 28°C, with a control plate maintained separately.

The experiment was conducted in triplicate. Radial mycelial growth of the target pathogen was measured once the control plate reached full growth. The percentage inhibition of radial mycelial growth was calculated using Vincent's formula (1947).

***In vitro* Evaluation of Botanicals**

The antifungal efficacy of various botanicals against *E. japonica* were assessed *in vitro* using the poisoned food technique with three replications for each botanical at concentrations of 5.00, 10.00 and 15.00 per cent. Eleven plant species, including turmeric, ginger, *Gliricidia*, *Bougainvillea*, *Eucalyptus*, neem, Pongamia, *Lantana*, papaya, *Calotropis* and garlic were selected for the study. At GKVK, Bengaluru fresh leaves or bulbs were collected from different locations. The plant materials were first washed thoroughly with tap water, then rinsed multiple times with distilled water and surface sterilized using 1.0 per cent sodium hypochlorite. After sterilization, 100 grams of each leaf or bulb material were chopped into small pieces and grounded using grinder. The resulting pulp was filtered through muslin cloth and stock solution of each plant extract was prepared and were used to evaluate their antifungal activity against *E. japonica*.

Results and Discussion

Isolation of the Pathogen

Panicles and leaves showing typical symptoms of udbatta or agarbattiroga were collected from a farmer's field. The pathogen, *E. japonica* was isolated from the diseased paddy panicles and was identified based on its colony characteristics, conidia and morphological traits. On potato dextrose agar (PDA) plates, the fungal growth appeared elevated, fluffy, with a leathery and waxy texture. The isolated pathogen was subsequently used for *in vitro* evaluation of fungicides, botanicals and bioagents.

***In vitro* Evaluation of Fungicides**

Copper oxychloride 50% WP, the non-systemic fungicide consistently inhibited mycelial growth at all three concentrations tested. Propineb 70% WP resulted in mean inhibition of 60.75 per cent while using Mancozeb 75% WP, a mean inhibition of 56.67 per cent was recorded. The inhibition percentages of other contact fungicides, such as Zineb 75% WP, Chlorothalonil 75% WP and Captan 50% WP, were 24.08, 39.26 and 41.80 per cent, respectively and were found less effective. All fungicides considerably reduced the test pathogen's growth at 1000 ppm concentration when compared to control with inhibition that ranged from 45.54 to 85.55 per cent. Copper oxychloride 50% WP was found to have highest efficacy of 85.55 per cent inhibition of mycelial growth, followed by Mancozeb 75% WP (74.46%) and Propineb 70% WP (66.68%). In contrast, Zineb 75% WP resulted in lowest inhibition of pathogen (17.79%), which remained similar evenat 500 ppm. At 250 ppm, Propineb 70% WP resulted in

the highest inhibition (53.35%), followed by Mancozeb 75% WP with 41.13% inhibition. Zineb 75% WP resulted in lowest inhibition at 8.91 per cent (Fig. 1 and Plate 1). Due to its high affinity of copperfor amino acids and carboxyl groups, copper reacts with proteins to block enzymes in target species and prevent the germination of fungal spores. Present findings are in accordance with those of Kumar (1998) and El-Naggar *et al.* (2015), who reported that copper oxychloride inhibited mycelial growth by 66.70 per cent.

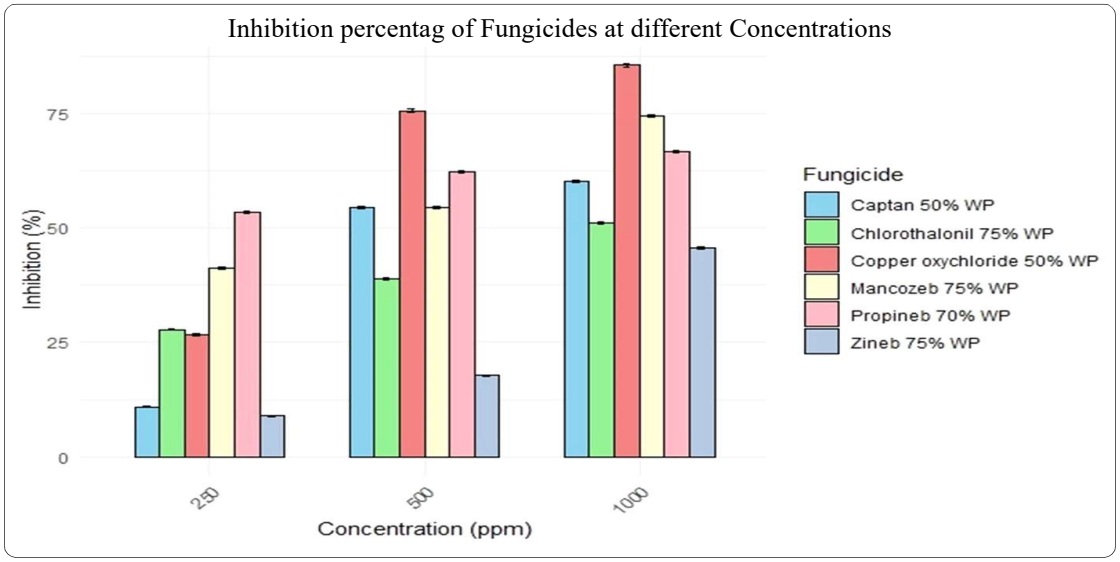


Fig. 1 : *In vitro* evaluation of contact fungicides against *Ephelis japonica*

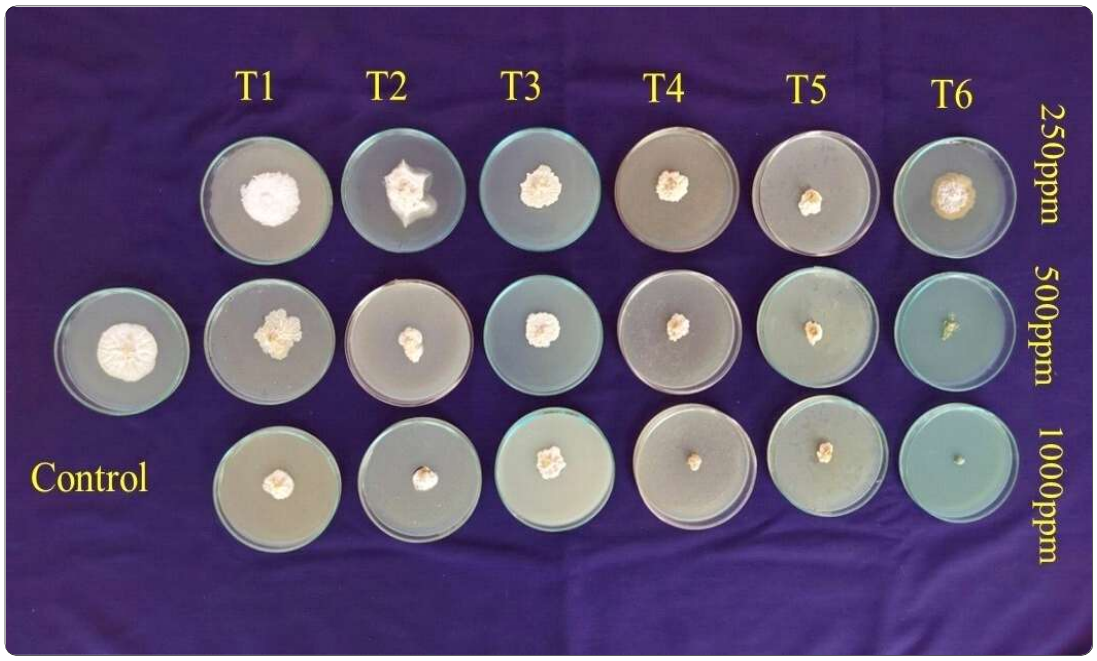


Plate 1 : *In vitro* evaluation of contact fungicides against *Ephelis japonica*

T_1 - Zineb 75% WP; T_2 - Captan 50% WP; T_3 - Chlorothalonil 75% WP; T_4 - Mancozeb 75 % WP; T_5 - Propineb 70 % WP; T_6 - Copper oxychloride 50 % WP

The cumulative mycelial inhibition of *E. japonica* caused by systemic fungicides, based on mean values, revealed that Tebuconazole 45 per cent SC resulted in 100 per cent mycelial inhibition at all three concentrations tested. It was followed by Hexaconazole 5% SC with 88.90 per cent inhibition, and Propiconazole 25% EC with 77.06 per cent inhibition. The remaining systemic fungicides, including Difenconazole 25% WP, Carbendazim 50% WP and Azoxystrobin 25% SC, were less effective with mycelial inhibition of 65.95, 63.72 and 50.00 per cent, respectively (Fig. 2 and Plate 2). Tebuconazole, a Demethylation Inhibiting (DMI) fungicide, affects the fungal cell wall by inhibiting

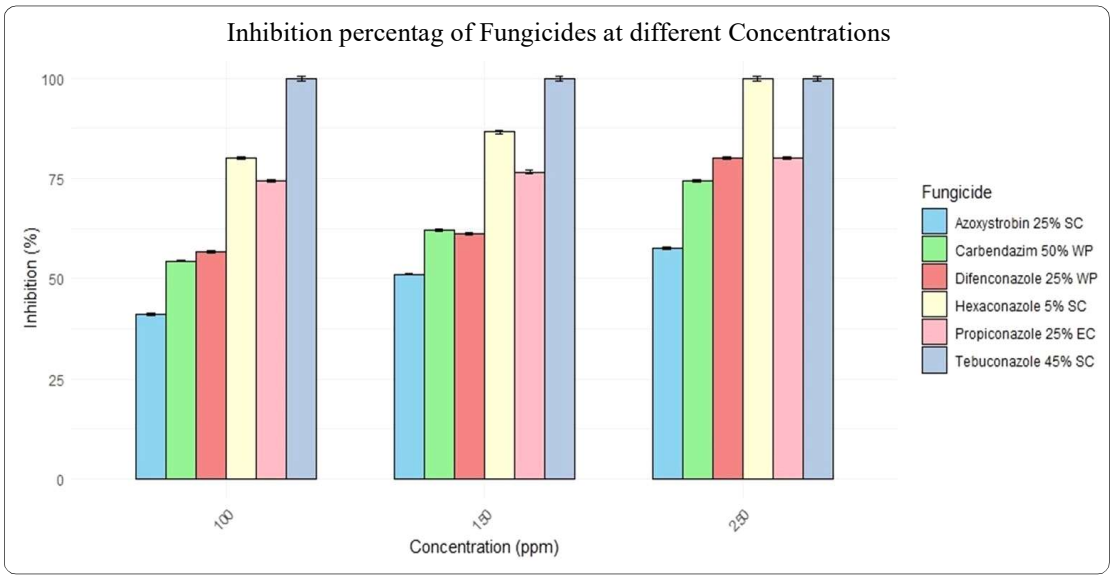


Fig. 2 : *In vitro* evaluation of systemic fungicides against *Ephelis japonica*

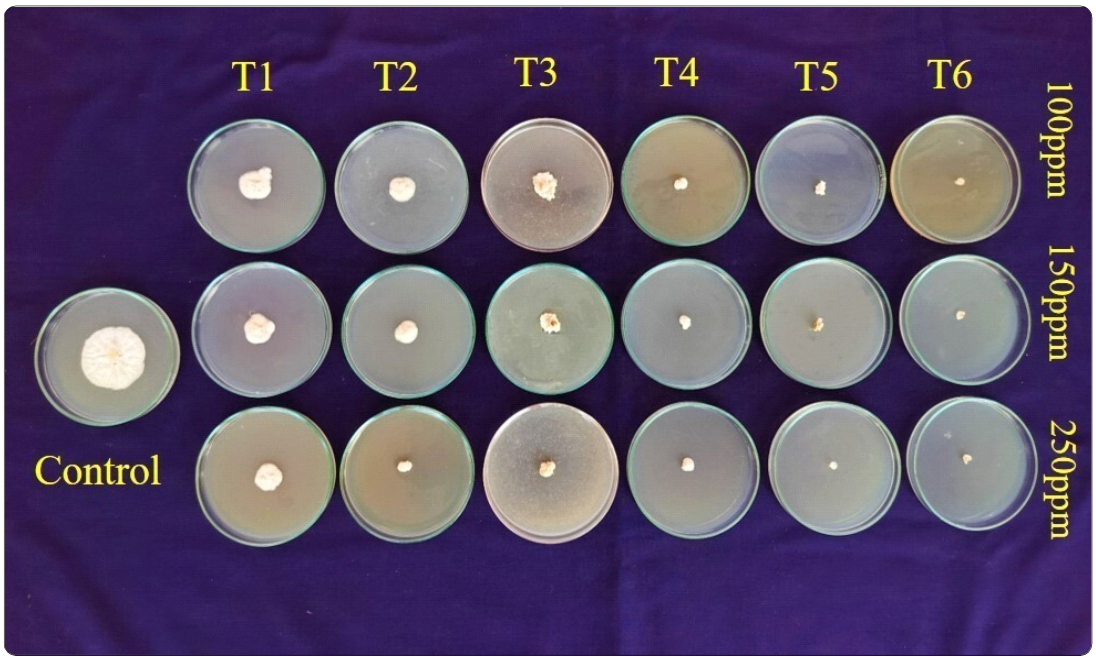


Plate 2 : *In vitro* evaluation of systemic fungicides against *Ephelis japonica*

T_1 - Azoxystrobin 25 % SC; T_2 - Difenconazole 25% WP; T_3 - Carbendazim 50 % WP; T_4 - Propiconazole 25 % EC; T_5 - Hexaconazole 5 % SC; T_6 - Tebuconazole 45 % SC

spore germination and interfering with ergosterol biosynthesis, an essential component for building the fungal cell wall. These findings are similar with earlier studies by Indrasenan *et al.* (1981), Sannegowda (1980), Sannegowda and Pandurangegowda (1986), Narayan (2014), Raji *et al*. (2016) and Edun *et al.* (2019) who reported that Tebuconazole as highly effective in controlling this pathogen.

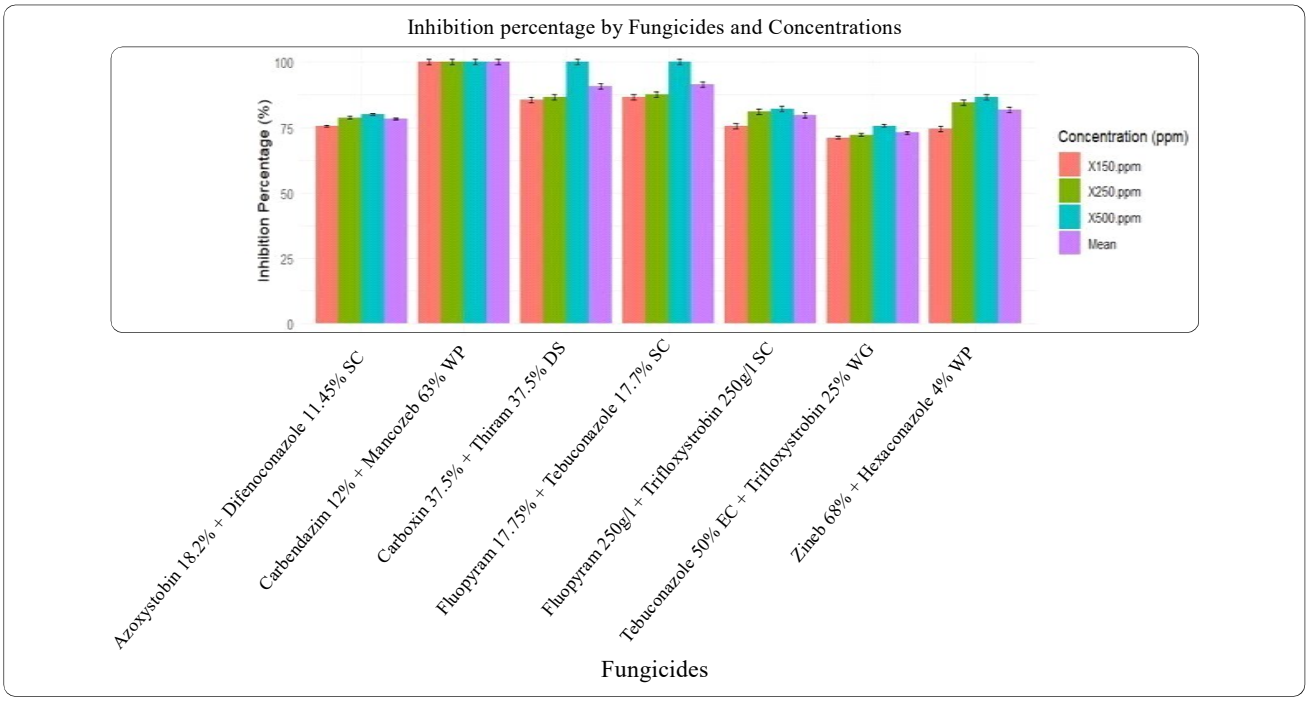


Fig. 3 : *In vitro* evaluation of combination products against *Ephelis japonica*

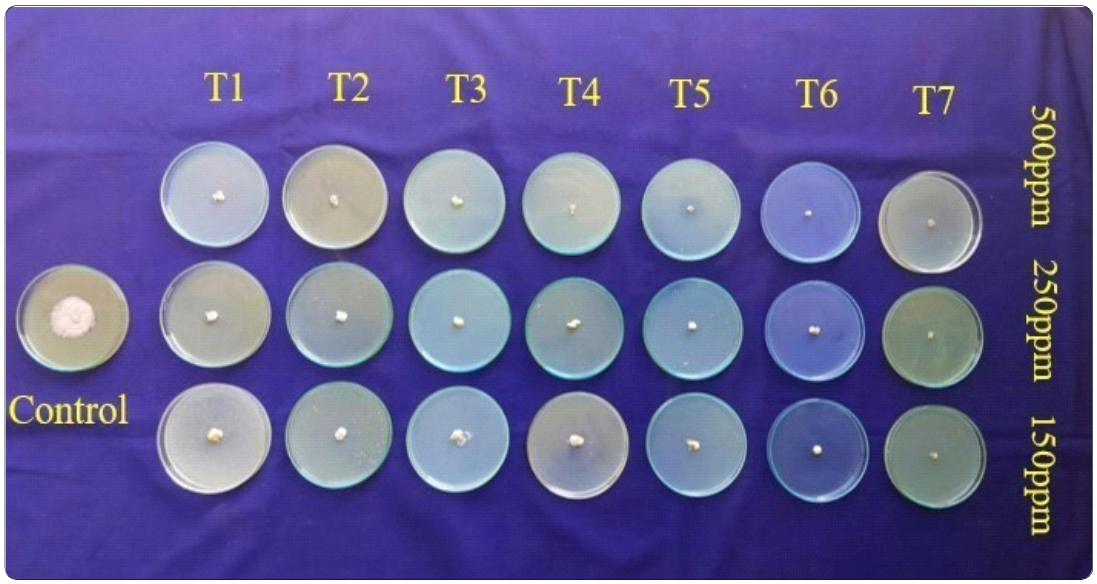


Plate 3 : *In vitro* evaluation of combination products against *Ephelis japonica*

T_1 - Tebuconazole 50 % EC + Trifloxystrobin 25 % WG; T_2 - Azoxystrobin 18.2 % + Difenoconazole 11.45 % SC; T_3 – Fluopyram 250g/l + Trifloxystrobin 250g/l SC; T_4 - Zineb 68 % + Hexaconozole 4 % WP; T_5 - Carboxin 37.5 % + Thiram 37.5 % DS; T_6 - Fluopyram 17.7 % + Tebuconazole 17.7 % SC; T_7 - Carbendazim 12 % + Mancozeb 63 %

The overall mean mycelial inhibition of *E. japonica* using combination product fungicide Carbendazim 12% + Mancozeb 63% WP was 100% at all three concentrations tested. It was followed by Carboxin 37.5% + Thiram 37.5% DS and Fluopyram 17.7% + Tebucona zole 17.7% SC treatments, with mean inhibition percentages of 90.72 and 91.47 per cent, respectively and were on par to one another. The least inhibition was noticed with Tebuconazole 50% EC + Trifloxystrobin 25% WG, (73.00% inhibition) (Fig. 3 and Plate 3). The combination of systemic and non-systemic fungicides in these products offered superior results, as they are of multiple sitesand different modes of action that prevented the development of fungal resistances trains to these systemic fungicides. These findings are similar with studies by Mohanty (1971), Kumar *et al*. (2016) and Ranganathaiah (1985), who also reported that Carbendazim + Mancozeb provided the highest inhibition of the pathogen.

In vitro Evaluation of Seed Treatment with Fungicides in Rolled Towel Method

The cumulative percentage of seed infection in paddy seeds treated with fungicides revealed that Mancozeb 63% + Carbendazim 12% WP resulted in lowest seed infection rate (1.00%) across all the tested concentrations. This was followed by Carboxin 37.5% + Thiram 37.5% DS (4.80%), Carbendazim 50% WP (4.88%), Tebuconazole 45% SC (22.26%), Mancozeb 75% WP (33.84%) and Copper oxychloride 50% WP (40.91%). In contrast, the control group exhibited the highest seed infection rate (85.59%) (Table 1).

The cumulative germination percentage of paddy seeds treated with Mancozeb 63% + Carbendazim 12% WP brought outthe highest germination rate (99.02%), followed by Carboxin 37.5% + Thiram 37.5% DS (98.41%) and Carbendazim 50% WP (98.12%), while withTebuconazole 45% SC, the germination rate was 92.67% as against the control with the lowest germination rate (41.16%) (Table 2).

The highest seedling vigor index (1965.85) was recorded when treated with Mancozeb 63% + Carbendazim 12% WP and was followed by Carboxin 37.5% + Thiram 37.5% DS (1784.70) and Carbendazim 50% WP (1779.13) as against the lowest vigor index in control (211.55) (Table 2). In Mancozeb 63% + Carbendazim 12% WP treated plants, the

Table 1

Evaluation of seed treatment fungicides on per cent seed infection in rolled towel method

Treatment	Fungicides	Per cent Seed infection			
		Concentration (%)			
		0.1	0.2	0.3	Mean
1	Copper oxychloride 50 % WP	46.39 (42.93) *	40.62 (39.59)	35.73 (36.71)	40.91 (39.76)
2	Mancozeb 75 % WP	40.05 (39.26)	34.37 (35.89)	27.11 (31.38)	33.84 (35.57)
3	Tebuconazole 45 % SC	28.10 (32.01)	22.06 (28.01)	16.64 (24.07)	22.26 (28.15)
4	Carboxin 37.5 % + Thiram 37.5 % DS	8.37 (16.82)	5.04 (12.97)	1.00 (5.74)	4.80 (12.66)
5	Carbendazim 50 % WP	8.69 (17.14)	5.63 (13.73)	0.34 (3.34)	4.88 (12.76)
6	Mancozeb 63 % + Carbendazim 12% WP	3.00 (9.97)	0.00 (0.00)	0.00 (0.00)	1.00 (5.74)
7	Control			85.59 (67.69)	
		Fungicides (F)	Concentration (C)		F×C
S.Em.±		0.39	0.28		0.67
C.D. @ 1%		1.13	0.72		1.94

*Figures in parenthesis are arcsine transformed values; Number of replications - 3

TABLE 2

Evaluation of seed treatment fungicides on germination percentage and seedling vigour index in rolled towel method

Treatment	Fungicides	Germination percentage				Seedling vigour index			
		Concentration (%)				Concentration (%)			
		0.1	0.2	0.3	Mean	0.1	0.2	0.3	Mean
1	Copper oxychloride 50% WP	84.10 (66.50) *	85.72 (67.80)	87.42 (69.23)	85.74 (67.81)	596.80	762.31	1062.63	807.24
2	Mancozeb 75% WP	87.66 (69.43)	88.50 (70.18)	91.78 (73.34)	89.31 (70.92)	734.56	851.78	1319.23	968.52
3	Tebuconazole 45% SC	89.44 (71.04)	92.13 (73.71)	96.46 (79.16)	92.67 (74.29)	855.27	1361.64	1715.23	1310.7
4	Carboxin 37.5% + Thiram 37.5% DS	95.46 (77.70)	99.61 (87.25)	100 (90.05)	98.41 (82.76)	1489.45	1827.22	2037.44	1784.7
5	Carbendazim 50% WP	96.02 (78.49)	98.35 (82.62)	100 (90.05)	98.12 (82.12)	1523.49	1883.24	1924.67	1779.13
6	Mancozeb 63% + carbendazim 12% WP	97.39 (80.70)	99.69 (86.81)	100 (90.05)	99.02 (84.32)	1863.59	1941.82	2092.14	1965.85
7	Control			41.16 (39.91)				211.55	
	Fungicides (F)	Concentration (C)	F×C	Fungicides (F)	Concentration(C)	F×C			
	S.Em.±	0.87	0.66	1.53	18.31	13.05	30.02		
	C.D. @ 1%	2.48	1.89	4.39	52.54	36.45	86.43		

*Figures in parenthesis are arcsine transformed values; Number of replications - 3

longest seedlings were produced (20.03 cm), followed by Carboxin 37.5% + Thiram 37.5% DS (18.19 cm) and Carbendazim 50% WP (18.00 cm) treatments. Other treatments that included Tebuconazole 45% SC (13.81 cm), Mancozeb 75% WP (10.69 cm) and Copper oxychloride 50% WP (9.25 cm), with the control group the shortest seedling length (5.50 cm) was observed (Table 3, Plate 4).

TABLE 3

Evaluation of seed treatment fungicides on seedling length in rolled towel method

Treatment	Fungicides	Seedling length (cm)			
		Concentration (%)			
		0.1	0.2	0.3	Mean
1	Copper oxychloride 50 % WP	7.04	8.83	11.90	9.25
2	Mancozeb 75 % WP	8.58	9.65	13.85	10.69
3	Tebuconazole 45 % SC	9.67	14.87	16.89	13.81
4	Carboxin 37.5 % + Thiram 37.5 % DS	15.24	18.27	21.07	18.19
5	Carbendazim 50 % WP	15.39	19.64	18.98	18.00
6	Mancozeb 63 % + Carbendazim 12 % WP	19.00	19.85	21.24	20.03
7	Control	5.50			
		Fungicides (F)	Concentration (C)	F×C	
	S.Em.±	0.73	0.67	1.20	
	C.D. @ 1%	2.18	2.05	3.52	

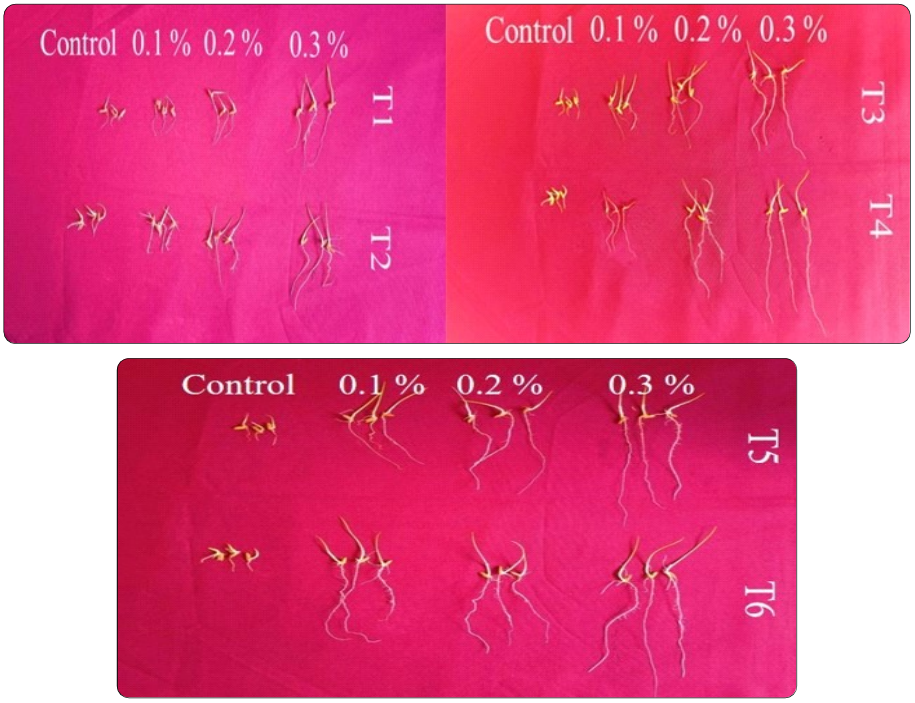


Plate 4 : Evaluation of seed treatment fungicides on seedling vigour in rolled towel method

T_1 - Copper oxychloride 50 % WP; T_2 - Mancozeb 75 % WP; T_3 - Tebuconazole 45 % SC; T_4 - Carboxin 37.5 % + Thiram 37.5 % DS; T_5 - Carbendazim 50 % WP; T_6 - Mancozeb 63 % + Carbendazim 12 % WP

As udbatta is a seed borne disease, as reported by Mohanty (1975), Govindu and Thirumalachar (1961), Shivanandappa and Govindu (1977), Ranganathaiah (1985), Mohanty (1979) and Kumar *et al.* (2016) demonstrated that seeds treated with Carbendazim at 4 g/kg exhibited the lowest incidence of udbatta disease, followed by a combination of Carbendazim 25% + Mancozeb 50% WS. The present results are consistent with the earlier studies. Udbatta being a seed borne disease, early management through chemical seed treatments is crucial for effective control.

In vitro Efficacy of Biocontrol Agents Against *E. Japonica* using Dual Culture Technique

Eco-friendly management of udbatta disease is achievable through the effective use of biocontrol agents. Among the eight bioagents tested against *E. japonica*, both *T. harzianum* and *T. hamatum* isolates resulted in 100 per cent inhibition and *T. harzianum* (Th-44) being particularly potent, effectively inhibited pathogen growth within three days of inoculation. With *T. viride* isolates, mycelial inhibition rates of 86.70% (Tv-2) and 82.8% (Tv-M) was observed. *Pseudomonas fluorescens* inhibited pathogen up to 83.00%, while *B. subtilis* it was 79.52 per cent inhibition. The lowest inhibition was observed with *T. asperellum* (74.20%), (Table 4 and Plate 5). Similar results were reported by Narayan (2014), who discovered that *B. subtilis* showed 80.26 per cent inhibition while *T. harzianum* completely over grew the pathogen, obtaining 100 per cent mycelial inhibition. Baite and Prabhukarthikeyan (2022) reported that *Trichoderma harzianum* and *B. subtilis* significantly inhibited the mycelial growth of claviceptacean fungi like *U. virens* in rice, achieving inhibition rates of 72.58 per cent and 65.89 per cent, respectively. The present findings also align with research conducted by Andargie *et al*. (2017) and Maurya *et al*. (2021).

In vitro efficacy of Volatile Organic Compounds (VOCs) Produced by Fungal Biocontrol Agents Against *Ephelis japonica* using Double Petri Dish Assay

Inverted plate technique or Double Petri dish assay was performed to assess the inhibitory effect of volatile released by *Trichoderma* sp. against *Ephelis japonica*. In the double Petri dish assay, *T. harzianum* (Th-41) caused the highest mycelial inhibition (77.24 %), followed by *T. viride* (Tv-2) (73.68 %). *Trichoderma hamatum* (Th-14) and *Trichoderma viride* (Tv-M) resulted in moderate inhibition with 65.56 and 58.15 per cent, respectively. The least inhibition was noticed in *T. asperellum* (Ta-6) with

Table 4

***In vitro* efficacy of biocontrol agents against *Ephelis japonica* using dual culture technique**

Treatment No.	Bioagents	Isolates	Percentmycelial inhibition
1	*Trichoderma hamatum*	(Th-14)	100.00 (90.05) *
2	*Trichoderma harzianum*	(Th-41)	100.00 (90.05)
3	*Trichoderma harzianum*	(Th-44)	100.00 (90.05)
4	*Trichoderma viride*	(Tv-M)	82.80 (65.50)
5	*Trichoderma viride*	(Tv-2)	86.70 (68.61)
6	*Trichoderma asperellum*	(Ta-6)	74.20 (59.47)
7	*Bacillus subtilis*	(Bs- O)	79.52 (63.09)
8	*Pseudomonas fluorescens*	(Pf-M)	83.00 (65.65)
	S. Em±		0.43
	CD @1%		1.32

*Figures in parenthesis are arcsine transformed values; Number of replications - 3

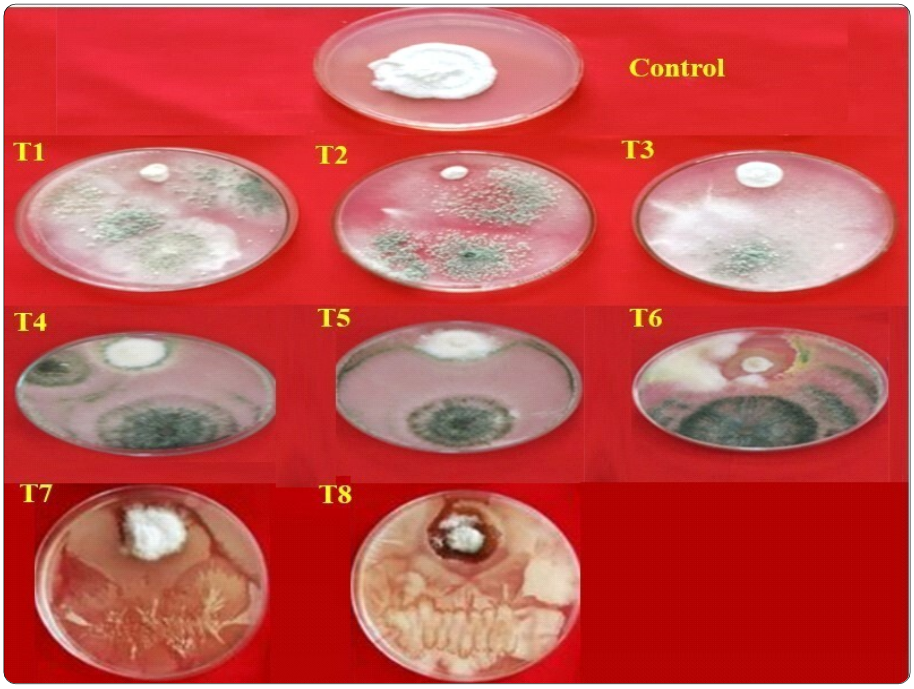


Plate 5 : *In vitro* efficacy of biocontrol agents against *E. japonica* using dual culture technique

T_1 - *Trichoderma hamatum* (Th-14); T_2 - *T. harzianum* (Th-41); T_3 - *T. harzianum* (Th-44); T_4 - *T. viride* (Tv-M); T_5 - *T. viride* (Tv-2); T_6 - *T. asperellum* (Ta-6); T_7 - *Bacillus subtilis* (Bs-O); T_8 - *Pseudomonas fluorescens* (Pf-M)

54.44 per cent (CD at 1% = 0.59). These results indicated that the volatiles produced by *Trichoderma* species effectively inhibit the growth of *E. japonica* (Fig. 4 and Plate 6).

The results of this study align with previous findings on the effectiveness of *Trichoderma* sp. against *U. virens* by inverted plate assay. Narayan (2014) reported that *T. harzianum* and *B. subtilis* significantly

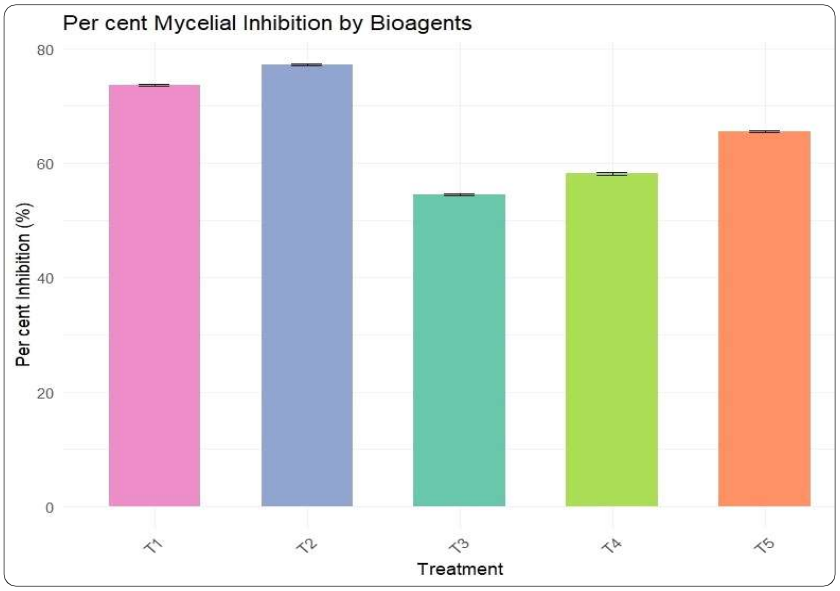


Fig. 4 : *In vitro* efficacy of VOCs produced by fungal biocontrol agents against *Ephelis japonica* using double Petri dish assay or inverted plate technique: T_1: *Trichoderma viride* (Tv-2); T_2: *T. harzianum* (Th-41); T_3: *T. asperellum* (Ta-6); T_4: *T. viride* (Tv-M); T_5: *T. hamatum* (Th-14)

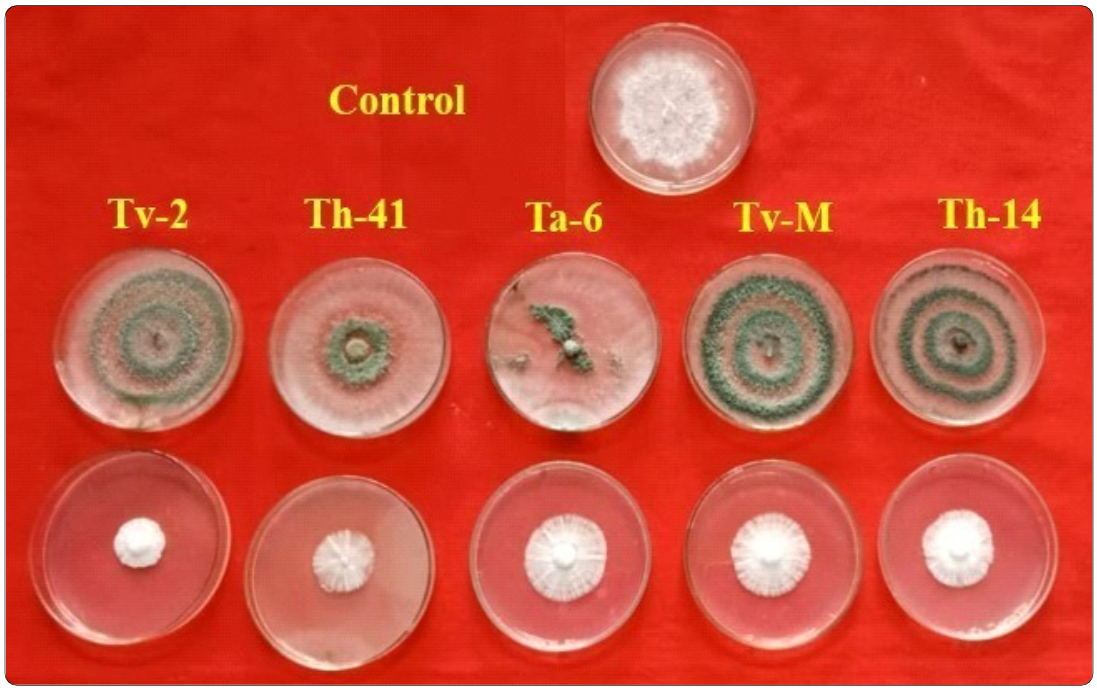


Plate 6 : *In vitro* evaluation of the efficacy of VOCs produced by fungal biocontrol agents against *Ephelis japonica* using double Petri dish assay: Tv-2 (*Trichoderma viride*-2); Th-41 (*T. harzianum*-41); Ta-6 (*T. asperellum*); Tv-M (*T. viride*-M); Th-14 (*T. hamatum*-14)

inhibited the mycelial growth of claviceptacean fungi like *U. virens* in rice, achieving inhibition rates of 72.58 per cent and 65.89 per cent, respectively.

The overall results indicate that the efficacy of biocontrol agents against fungal pathogens varies depending on the isolate and the evaluation method. *Trichoderma* sp., particularly *T. harzianum* and *T. hamatum* showed strong antagonistic effects in the dual culture technique. Mean while, *T. viride* and *T. harzianum* demonstrated high efficacy through volatile emissions in the double Petri dish assay. Per cent inhibition was greater in dual culture technique than in inverted plate assay because the inverted plate technique relies solely on volatile compounds for inhibition. In contrast, the dual culture technique allows *Trichoderma* to compete with the pathogen for resources directly, byproducing antimicrobial compounds and by parasitizing the pathogen, leading to more effective inhibition.

In vitro Evaluation of Botanical Extracts Against *Ephelis japonica*

The experiment assessed the fungitoxic effects of various plant extracts on the mycelial growth of *Ephelis japonica* at three concentrations: 5, 10 and 15 per cent. The inhibitory effects of these extracts varied significantly. As indicated in Table 5 and Plate 7, all plant extracts reduced pathogen growth to different extents at the 5 per cent concentration, with inhibition ranging from 10.47 to 68.55 per cent. The highest inhibition was achieved with garlic bulb extract (68.55%), followed by turmeric rhizome extract (64.55%) and neem leaf extract (52.77%). In contrast, the leaf extract of *Calotropis* exhibited the lowest inhibition (10.47%).

At 10 and 15 per cent concentrations, inhibition ranged from 18.33 to 75.24 per cent and 26.47 to 88.47 per cent, respectively, with a similar trend observed across concentrations. The mean inhibition of mycelial growth across all concentrations ranged from 19.33 to 77.18 per cent. The bulb extract of *Allium sativum* showed the highest average inhibition (77.18%), followed by the rhizome extract of turmeric (72.33%). Leaf extracts of neem, *Eucalyptus*, ginger, papaya and curry leaves also demonstrated significant inhibitory effects, with inhibition rates ranging from 64.06 to 42.12 per cent. Consistently, *Calotropis* leaf extract exhibited the lowest inhibition (19.33%).

TABLE 5

In vitro* evaluation of botanical extracts against *Ephelis japonica

Treatment No.	Botanicals	Percent inhibition over control			
		Concentration (%)			
		(C_3) 5%	(C_2) 10%	(C_1) 15%	Mean
1	*Allium sativum* (Garlic)	68.55# (64.57) *	75.24 (54.91)	88.47 (69.45)	77.18 (61.47)
2	*Curcuma longa* (Turmeric)	64.55 (62.41)	71.11 (68.96)	82.57 (78.91)	72.33 (72.03)
3	*Azadirachta indica* (Neem)	52.77 (54.91)	67.92 (72.78)	73.08 (72.68)	64.06 (36.42)
4	*Eucalyptus globulus Labill.* (Eucalyptus)	48.29 (55.19)	56.71 (59.37)	69.33 (75.90)	58.84 (85.00)
5	*Zingiber officinale* L. (Ginger)	42.18 (54.76)	51.98 (62.06)	62.09 (58.56)	51.33 (85.50)
6	*Carica papaya* (Papaya)	38.48 (49.92)	48.44 (53.48)	58.66 (65.39)	46.86 (62.70)
7	*Murraya koenigii* (Curry leaves)	37.75 (34.62)	44.57 (34.88)	57.09 (89.92)	42.12 (44.36)
8	*Bougainvillea glabra* (Bougainvillea)	32.44 (37.73)	39.33 (41.02)	42.00 (48.45)	37.66 (51.94)
9	*Gliricidia maculata (Kunth) Steud* (Gliricidia)	29.11 (39.88)	34.72 (46.91)	38.22 (73.98)	33.94 (45.57)
10	*Lantana camara* (Lantana)	18.77 (39.10)	29.22 (43.98)	37.00 (21.94)	28.65 (52.40)
11	*Calotropis procera* (Calotropis)	10.47 (10.96)	18.33 (12.90)	26.47 (16.86)	19.33 (40.01)
	Mean	40.16 (39.32)	54.36 (47.50)	62.47 (52.22)	
		Botanicals (B)	Concentration (C)	Interaction BxC	
	S. Em.±	0.53	0.67	1.56	
	CD@ 1%	1.62	1.70	3.47	

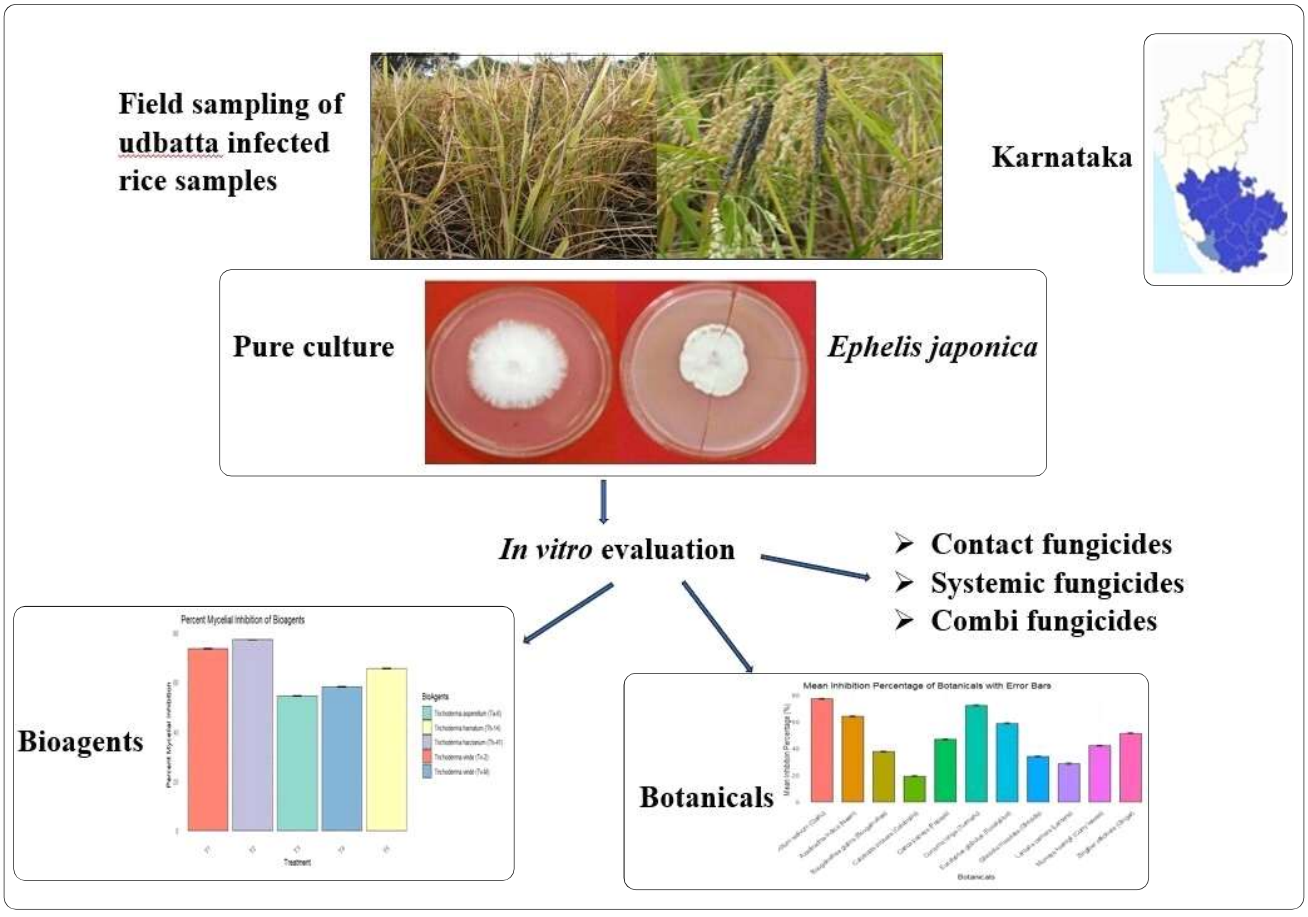
*Figures in parenthesis are arcsine transformed values; Number of replications- 3

T_1 - Garlic; T_2 - Turmeric; T_3 - Neem; T_4 - *Eucalyptus;* T_5- Ginger; T_6 - Papaya;
T_7 - Curry leaves; T_8 - *Bougainvillea;* T_9 - *Gliricidia;* T_{10} - *Lantana* and T_{11} - *Calotropis*

Plate 7 : *In vitro* evaluation of botanicals against *Ephelis japonica*

These findings align with previous studies by Raji *et al*. (2016), Zheng *et al*. (2019), Maurya *et al*. (2021) and Sumon *et al*. (2022), which reported similar antifungal properties in these plants. The antimicrobial activity of garlic is attributed to allicin, which interacts with thiol groups in enzymes, disrupting cysteine proteinase metabolism-a critical factor in the virulence of *E. japonica* (Narayan, 2014). Similarly, the antifungal efficacy of turmeric, attributed to curcumin, has been well-documented (Lengai *et al*., 2020). The relatively lower inhibition by *Calotropis procera* (19.33%) could be due to the pathogen's increased virulence and environmental factors, as suggested by Mahmud *et al*. (2017) and White *et al.* (1995).

In conclusion, *Ephelis japonica*, the seed-borne pathogen responsible for udbatta disease in rice, poses a significant threat to crop health. This *in vitro* screening study identified Copper oxychloride 50% WP and Propineb 70% WP as effective non-systemic fungicides, while systemic fungicides such as Tebuconazole 45% SC and Hexaconazole 5% SC also demonstrated substantial inhibition of the pathogen's radial growth. Combination products like Mancozeb 63% + Carbendazim 12% WP and Fluopyram 17.7% + Tebuconazole 17.7% SC yielded promising results as well. The rolled towel method was employed to assess the seed-borne nature of the pathogen and the efficacy of various fungicides. Among these, Mancozeb 63% + Carbendazim 12% WP and Carboxin 37.5% + Thiram 37.5% DS significantly reduced seed infection rates with the highest germination percentages, seedling length and vigor indices. Out of all the plant extracts evaluated, Garlic bulb extract was recorded to have the greatest pathogen inhibition (77.18%), while *Calotropis*



Schematic representation of sampling of infected udbatta rice panicles, screening of fungicides, botanicals and bioagents against *Ephelis japonica*

procera leaf extract with the least pathogen inhibition (19.33%). Among bioagents, *T. harzianum* and *T. hamatum* showcased complete inhibition (100%), while *T. asperellum* depicted comparatively lower inhibition (74.20%). Seed borne nature of *E. japonica* causes seed abortion, seed rot, necrosis, sclerotization of seeds, reduced or lost germination potential, seedling damage and disease development during later stages of plant growth due to systemic or localized infections. The pathogen is transmitted through infected seeds during sowing, resulting in systemic infection. The infected seeds serve as the primary inoculum, allowing the pathogen to persist between cropping seasons. Infected seeds can be detected using the rolled towel method or by observing mycelial growth on seed-testing media. To effectively manage and control *E. japonica*-associated udbatta disease, further field-level studies are essential to assess the efficacy of fungicide seed treatments, particularly their impact on seed infection and overall disease control strategies.

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