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Screening of Castor Germplasm and Efficacy Studies of Bioagents and Fungicides Against *Botryotinia ricini* (Godfrey) Whetzel

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Castor is an important non-edible oilseed crop having exceptional oil characteristics for the chemical industry. The crop hosts several diseases, among them gray mold is the most destructive one hindering its productivity. This article presents a study assessing the resistance of different germplasm lines screened against *Botrytis ricini* under artificial conditions. It also examines the effect of various fungicides on *B. ricini* isolates, which were obtained from infected *Ricinus communis* (castor oil plant) using standard tissue isolation techniques. The fungus was isolated from infected Castor raceme by standard tissue isolation technique and its pathogenicity was established by proving Koch's postulates. Out of 47 germplasm lines screened, TSIL-19 showed resistance response to gray mold under both detached spike and capsule method. Under *in vitro* assessment, *Trichoderma viride* (86.11 %) and *Pseudomonas fluorescens* (71.48 %) bio agents exhibited maximum mycelial growth inhibition of the pathogen. Among the seven combination fungicides evaluated, Fenamidone 10% + Mancozeb 50% WG recorded highest mycelial growth inhibition (100%) of *B. ricini.*

Keywords: Castor; Botryotinia ricini; grey mould; germplasm; resistance; fungicides.

1. INTRODUCTION

Castor (*Ricinus communis* L., 2n = 20) is the most important non-edible oilseed crop of arid and semi-arid regions grown across the tropical, sub-tropical and warm temperate regions of the world. It belongs to Euphorbiaceae family, is indigenous to Eastern Africa and originated in Ethiopia which contains 40-60 per cent oil content [1]. Castor oil stands unique among the other vegetable oils because of the presence of ricinoleic acid, a hydroxyl fatty acid contributing to high specific gravity and thickness compared to other vegetable oils, with a number of uses [2]. Castor oil is rich in triglycerides, mainly ricinolein, which is used predominantly for pharmaceutics, manufacturing of drugs and it possesses exceptional oil features for chemical industries and its global demand is constantly rising at 3–5 per cent per annum [3].

India is the world's largest producer of castor seed and oil, exports shipments of 5.72 lakh metric tons of castor oil [4]. In India, castor cultivation accounts to an area of 9.92 lakh hectares with 19.61 lakh tons of castor oil production [4]. Gujarat, Rajasthan, Karnataka, Orissa and Andhra Pradesh are the prominent castor growing states of India. Gujarat stands first in castor area and oil production in the country accounting for 7.41 lakh hectares with 16.59 lakh tons castor oil production and also leading in terms of productivity with 2231 kg/ha of yield [5,6].

Generally, the castor plant is infected by several pathogens such as fungi, bacteria, virus, mycoplasma, and nematode leading to 100 per cent yield losses and poor market value of seeds. In India alone, about 80 per cent of yield loss in castor crop is attributed to fungal disease [7]. Regardless of the tolerance ability of the castor plant to biotic stresses, gray mold, vascular wilt and charcoal rot, are the major diseases that is undermining its productivity [8]. Among these, gray mold is the most devastating and difficult disease to manage. It causes direct damage to castor capsules and inflorescence. Under high rainfall and temperature of 25°C high disease severity was observed [9-12]. Due to gray mold yield loss could be as high as 100 per cent [13]. Castor gray mold is polycyclic. Hence, infection can reoccur many times when the pathogen is blown by air to a new site in a season.

The *B. ricini* (Godfrey) is the fungal pathogen that causes gray mold in castor and its anamorphic phase is *Amphobotrys ricini* [14]. The anamorphic form of castor gray mold is capable of causing an epidemic under favourable environmental conditions [15]. In India, gray mold of castor was first reported in Karnataka [16] and appeared as an epidemic in 1985, the pathogen was identified as *B. ricini* [17]. During Kharif 1987, gray mold occurred in an epidemic form causing extensive damage to the crop in erstwhile Andhra Pradesh [18] and Tamil Nadu [19] which led to the decline in castor cultivation. Due to gray mold, castor area is reducing in the states of Gujarat, Rajasthan, Andhra Pradesh, Tamil Nadu, Karnataka and Odisha. Disease appears year after year attaining serious proportions limiting castor production in southern states of India [20].

A detailed study on castor gray mold was carried out during 20th century [21]. However, the contemporary studies on castor gray mold pathogen for its physiology, management has to be levitated for its control [22]. The identification of resistant castor cultivars, coupled with the deployment of suitable management methods, will significantly improve yield, pest and disease resistance, and overall sustainability for castor breeders and growers globally.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Pathogen

The plants showing typical symptoms of gray mold such as bluish spots on capsules from which yellow liquid oozes out and racemes covered by tan to gray coloured fungal growth, caused by *B.ricini* were collected from fields of K-7 block, ZARS, GKVK, University of Agricultural Sciences, Bengaluru. Gray mold infected capsules were microscopically examined for confirmation of the fungus. Sections of the diseased capsules were made with the help of a sharp blade on a clean glass slide having a drop of lactophenol. The specimen was then covered with a cover slip and observed under compound microscope. After confirmation of the fungus as *Botryotis*, infected capsules exhibiting typical gray mold symptoms were selected and pathogen was isolated by following standard tissue isolation method [23,24].

2.2 Isolation and Purification of Pathogen

The infected capsules were cut into small pieces (5-10 mm long), and were surface sterilized by immersing in 1 per cent sodium hypochloride for 2 minutes and then rinsed twice in sterilized distilled water and placed on to the oat meal agar added with streptomycin [24]. The plates were incubated upside down at 23±1˚C temperature for 7 days. The fungal growth emerging from diseased capsule pieces were observed. A loop full of fungal culture developed on oat meal agar medium in Petri plates was taken on a glass slide and observed under microscope for presence of conidia.

Single spore isolation technique was followed for the purification of the pathogen [25]. The spore suspension of the fungal isolate was prepared in sterile distilled water and 1 mL of the suspension from the fungal isolate was spread gently on 15 mL of molten 2 per cent water agar. Petri plates were observed for the presence of conidia under compound microscope after 10 days. The spore along with water agar was picked and transferred on oat meal agar medium plates and slants. Petri plates were incubated at room temperature (23±1˚C) and observed for fungal growth and the pure culture so obtained was preserved on oat meal agar medium slants in the refrigerator for further use.

2.3 Evaluation of Castor Germplasm Against Gray Mold of Castor

2.3.1 Screening techniques for resistance assessment under natural conditions

A total of 47 germplasm accessions which included DCH-519 as susceptible check and 48-1 (Jwala) as resistant check were evaluated against castor gray mold. The gray mold disease incidence was recorded in castor crop stand according to the diagrammatic scale of assessment (McKinney, 1923) and per cent disease incidence (PDI) was calculated.

The per cent disease index (PDI) was calculated by using (Vincent, 1947) infection index.

$$PDI\ (\%) = Sum\ \text{of individual ratings} / Total\ number\ \text{of spikes observed} \times Maximum\ disease\ \text{grade} \times 100$$

2.3.2 Screening techniques for resistance assessment under artificial conditions

2.3.2.1 Detached spike/ raceme technique

The 20 days old Spikes/ racemes along with 10 cm stalk were taken from castor plants. Cut end of stalks were immersed in 2 per cent sucrose solution in conical flasks and sprayed with spore suspension (10^6 conidia/mL) of *B. ricini*. The spikes were kept in glasshouse where humidity of 90 %, temperature of 27ºC and continuous capsule wetness were maintained by fogging. Screening of large number of castor germplasm/ breeding lines (Table. 1) against gray mold was done using the method [26].

2.3.2.2 Detached capsule technique

The 20 days old capsules were detached from castor spikes, surface sterilized and dipped in a spore suspension ((10^6 conidia/mL) of *B. ricini*. Inoculated capsules were maintained at 27ºC temperature and 90 per cent relative humidity. Wetness on capsules was maintained by spraying water at 8 h interval [27].

Table 1. List of germplasm used for screening against *B. ricini* infectingcastor

Sl. No.	Germplasm	Sl. No.	Germplasm	Sl. No.	Germplasm
1	DCH-519	18	BCG-23	35	TSIL-14
2	48-1	19	RG-2661 (GS)	36	TSIL-16
3	HCG-26	20	RG-2822	37	TSIL-17
4	MI-54	21	RG-3798 (OLD)	38	TSIL-18
5	MI-56	22	RG-2661-1	39	TSIL-19
6	MI-72	23	RG-2787	40	TSIL-20
7	MI-74	24	RG-109	41	TSIL-21
8	ICS-234	25	RG-72-1 (GS)	42	TSIL-22
9	ICS-245	26	TSIL-1	43	TSIL-23
10	ICS-248	27	TSIL-2	44	TSIL-24
11	ICS-258	28	TSIL-3	45	TSIL-25
12	ICS-270	29	TSIL-4	46	TSIL-26
13	ICS-272	30	TSIL-8	47	TSIL-27
14	ICS-272(NSP)	31	TSIL-9		
15	ICS-271-1	32	TSIL-10		
16	ICS-253	33	TSIL-11		
17	BCG-10	34	TSIL-13		

(sources AICRP on Castor and Directorate of Research, UAS, GKVK, Bangalore)

2.3.2.3 Host resistance assessment

Based on the infection levels of *B. ricini* on primary, secondary and tertiary racemes a 0-9 scale was developed by Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad was utilized here for resistance assessment, along with a diagrammatic scale [28].

2.3.3 Evaluation of bio agents and combination fungicides against gray mold of castor

2.3.3.1 In vitro evaluation of bio agents against B. ricini

Isolates of bacterial (*P. fluorescens*) and fungal bio control agents (*T. viride*) were used to evaluate the efficiency in reducing the growth of *B. ricini* through dual culture technique. Bio control agents and the *B. ricini* culture were placed side by side on a single Petri dish containing solidified PDA. There were three replications for each treatment with one control, later incubated at 25ºC and grown for 7 days. The diameter of the colony of both the biocontrol agent and the pathogen was measured in two directions and the average was calculated [29].

2.3.3.2 In vitro evaluation of fungicides against B. ricini

Poisoned food technique was employed for the evaluation of combination fungicides in the laboratory, Potato dextrose agar medium (100 mL) was prepared in the 250 mL conical flask. Required quantity of test fungicides were calculated and added to the sterilized medium each separately. Flasks containing poisoned medium were shaken well to have even and uniform distribution of the fungicides and about 20 mL of poisoned PDA was poured in to each of the respective labelled sterilized Petri plates and allowed to solidify. The solidified plates were then inoculated by 5 mm disc of one week old culture of *B. ricini,* with three replications. The control plates without fungicides were also inoculated and kept for incubation. Inoculated plates were incubated at 23±2°C. The observations on colony diameter were recorded after 7 days. The inhibition zone was calculated by using the following formula [29].

The percentage of inhibition was estimated using the following formula

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

Where,

I = Per cent inhibition,
C = Growth in control
T = Growth in treatment

2.3.3.3 In vitro evaluation of fungicides in inhibiting B. ricini on detached spikes of castor

Spikes/ racemes of 20 days old along with 10 cm stalk were taken from castor gray mold susceptible germplasm DCH-519, cut end of

stalks were immersed in 2 per cent sucrose solution in conical flasks and sprayed with a spore suspension (10^6 conidia/mL) of *B. ricini* one day prior to the fungicide application. The combi fungicides solution at concentration of 500 and 1000 ppm were sprayed to castor spikes and for positive control spray 1 mL of Propiconazole 250EC was sprayed. Three replications were maintained for each treatment. The spikes were kept in glasshouse where relative humidity of 90 per cent, temperature of 27ºC and continuous capsule wetness were maintained by fogging. The per cent inhibition of pathogen by combi products were calculated after 7 days of fungicidal spray and data were analyzed statistically.

2.4 Statistical Analysis

The data generated for different experiments were analyzed using Design of Experiments (DOE) was conducted using OPSTAT software (Statistical Package for Agricultural Scientists), developed by Chaudhary Charan Singh Haryana Agricultural University. Hisar and the inferences were made with a probability of one per cent for all laboratory experiments and a probability of five per cent for glass house experiments

3. RESULTS

3.1 Isolation and Morphological Identification of *B. ricini* from Capsules of Castor

The *B. ricini* was found in isolates prepared from naturally infected DCH-519 castor capsules with characteristic grey mould symptoms. The fungus formed irregular, fluffy, radial, or concentric ring colonies on oat meal agar medium. The colonies were originally hyaline to light brown in hue before turning dark grey over time. Conidia were generated on cylindrical, straight, pale brown conidiophores that were dichotomously branched. Microconidia were globose and hyaline, while macroconidia were unicellular, globose, and pale brown in hue. On day twelve following inoculation, little black sclerotia appeared around the Petri dish's edges. On oat meal agar medium, the fungus was grown pure and refined using the single spore isolation technique.

The fungus growing on oat meal agar media generated septate and branching mycelium, as well as dichotomously branched conidiophores that carried globose, single-celled conidia that ranged in colour from light brown to hyaline. These pathogenic isolates were identified in earlier research and were further utilized in the current study.

3.2 Evaluation of Castor Germplasm Against Gray Mold of Castor

The ideal, easiest, and most cost-effective method of disease management is the selection of resistant varieties for controlling plant diseases. This approach does not have any detrimental effects on the natural ecosystem; instead, it helps maintain good crop health and ensures potential yield. Therefore, identifying sources of resistance is essential in breeding programs. Consequently, 47 recognized castor germplasm lines were screened for resistance against gray mold. The results of the screening for gray mold incidence are as follows.

3.3 Screening Techniques for Resistance Assessment under Natural Conditions

Out of 47 germplasm lines screened against *B. ricini* under natural conditions, HCG-26, RG-72-1 (GS), TSIL-2, and TSIL-3 showed immune response as with 0 per cent disease incidence. Four germplasm lines namely ICS-248 (8.89 %), ICS-272(4.44 %), ICS- 253(6.67 %) and TSIL-19 (8.89 %) were resistant to the gray mold. Germplasm lines viz., ICS-234 (17.78 %), ICS - 272(NSP) (20 %), RG-2822 (15.56 %), TSIL-1 (11.11 %) and TSIL-4 (13.33 %) were moderately resistant. Germplasm lines viz., MI-54 (22.22 %), RG-2661(GS) (24.44 %), RG-2787 (26.67%) and TSIL-8 (28.89 %) were moderately susceptible. Sixteen germplasm lines namely TSIL-9 (46.67 %), TSIL-10 (44.44 %), TSIL-11 (48.89 %), TSIL-13 (42.22 %), TSIL-1 (40 %) , TSIL-16 (40 %) , TSIL-17 (48.89 %), TSIL-18 (42.22 %), TSIL-20 (46.67 %), TSIL-21 (40 %), TSIL-22 (48.89 %), TSIL-23 (42.22 %), TSIL-24 (48.89 %), TSIL-25 (46.67 %), TSIL-26 (46.67 %) and TSIL-27 (40 %) were susceptible to gray mold of castor. Twelve germplasm lines viz., MI-56 (100 %), MI-72 (91.11 %), MI-74 (86.67 %), ICS-245 (95.56 %), ICS-258 (95.56 %), ICS-270 (93.33 %), ICS-271-1 (82.22 %), BCG-10 (86.67 %), BCG-23 (91.11 %), RG-3798 (OLD) (95.56 %), RG-2661-1 (84.44 %) and RG-109 (68.89 %) were highly susceptible to *Botryotinia* gray mold incidence. Per cent disease incidence and reaction of different germplasm lines that are assessed for resistance under natural condition are depicted in Table 2, Fig. 1A and Fig. 2.

Table 2. Natural screening of castor germplasm against *B. ricini* infectingcastor

Sl. No.	Germplasm	PDI (%)	Grade	Disease reaction
1	HCG-26	0.00	0	I
2	MI-54	22.22	5	MS
3	MI-56	100.00	9	HS
4	MI-72	91.11	9	HS
5	MI-74	86.67	9	HS
6	ICS-234	17.78	3	MR
7	ICS-245	95.56	9	HS
8	ICS-248	8.89	1	R
9	ICS-258	95.56	9	HS
10	ICS-270	93.33	9	HS
11	ICS-272	4.44	1	R
12	ICS-272(NSP)	20.00	3	MR
13	ICS-271-1	82.22	9	HS
14	ICS-253	6.67	1	R
15	BCG-10	86.67	9	HS
16	BCG-23	91.11	9	HS
17	RG-2661 (GS)	24.44	5	MS
18	RG-2822	15.56	3	MR
19	RG-3798 (OLD)	95.56	9	HS
20	RG-2661-1	84.44	9	HS
21	RG-2787	26.67	5	MR
22	RG-109	68.89	9	HS
23	RG-72-1 (GS)	0.00	0	I
24	TSIL-1	11.11	3	MR
25	TSIL-2	0.00	0	I
26	TSIL-3	0.00	0	I
27	TSIL-4	13.33	3	MR
28	TSIL-8	28.89	5	MS
29	TSIL-9	46.67	7	S
30	TSIL-10	44.44	7	S
31	TSIL-11	48.89	7	S
32	TSIL-13	42.22	7	S
33	TSIL-14	40.00	7	S
34	TSIL-16	40.00	7	S
35	TSIL-17	48.89	7	S
36	TSIL-18	42.22	7	S
37	TSIL-19	8.89	1	R
38	TSIL-20	46.67	7	S
39	TSIL-21	40.00	7	S
40	TSIL-22	48.89	7	S
41	TSIL-23	42.22	7	S
42	TSIL-24	48.89	7	S
43	TSIL-25	46.67	7	S
44	TSIL-26	46.67	7	S
45	TSIL-27	40.00	7	S
46	DCH-519	95.00	9	HS
47	48-1	10.00	1	R

(Here the extent of disease symptoms on each plant, often scored on a scale (e.g., 0-5 or 0-9) based on visual assessment).

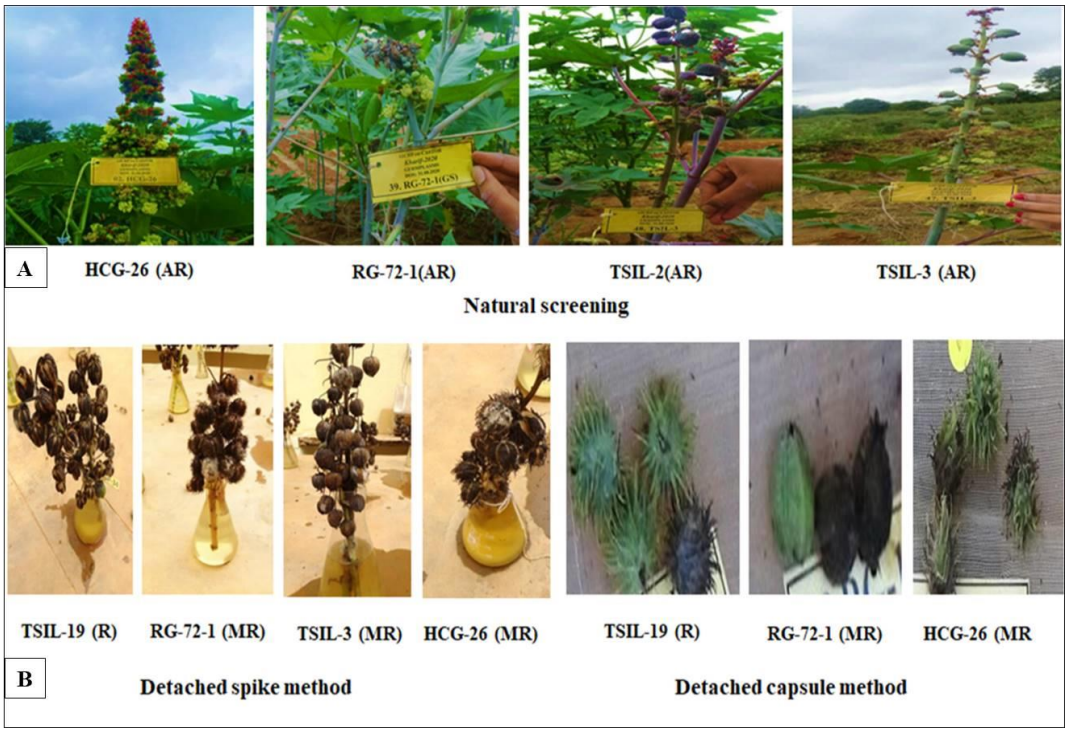

Fig. 1. A: Natural screening of castor germplasm against *B. ricini* infecting castor B: Artificial screening of castor germplasm against *B. ricini* infecting castor

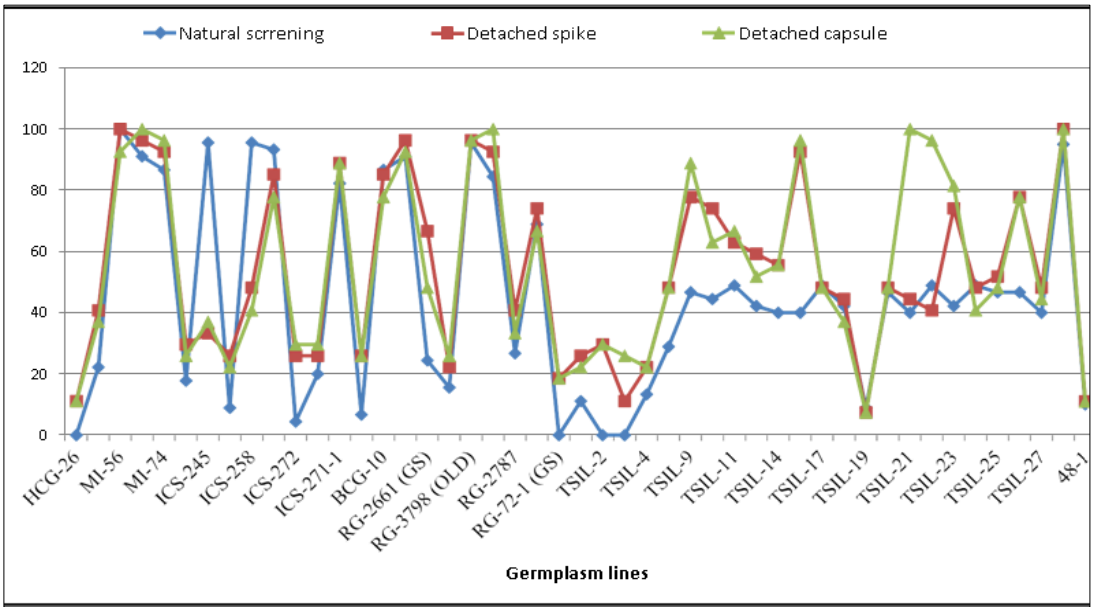

Fig. 2. Screening of castor germplasm against *B. ricini* infecting castor (in this figure Y axis denotes PDI % and X denotes all castor germplasm under this study)

3.4 Screening Techniques for Resistance Assessment under Artificial Conditions

Initial symptoms of gray mold infection appear 5 days after inoculation. By 7th day, all the capsules will be covered with cottony gray mycelium of the fungus in detached spike method. In detached capsule method, symptoms appeared on capsules at 4 days after inoculation. By 6th day capsules were fully covered with mycelium and severity was recorded for assessing the

resistance. Forty seven castor germplasm lines were screened for their reaction against *Botryotinia* gray mold under artificial conditions in glasshouse by using detached spike and detached capsule method. Out of 47 germplasm, TSIL-19 (7.41 %) showed resistance to the gray mold disease under both detached spike and capsule method (Table 3, Fig. 1B and Fig. 2).

Table 3. Artificial screening of castor germplasm against *Botryotinia ricini* infectingcastor

Sl. No.	Germplasm	Intensity of infection (%)					
		Detached spike method			Detached capsule method		
		PDI(%)	Grade	Reaction	PDI (%)	Grade	Reaction
1	HCG-26	11.11	3	MR	11.11	3	MR
2	MI-54	40.74	7	S	37.04	7	S
3	MI-56	100.00	9	HS	92.59	9	HS
4	MI-72	96.30	9	HS	100.00	9	HS
5	MI-74	92.59	9	HS	96.30	9	HS
6	ICS-234	29.63	5	MS	25.93	5	MS
7	ICS-245	33.33	7	S	37.04	7	S
8	ICS-248	25.93	5	MS	22.22	5	MS
9	ICS-258	48.15	7	S	40.74	7	S
10	ICS-270	85.19	9	HS	77.78	9	HS
11	ICS-272	25.93	5	MS	29.63	5	MS
12	ICS-272(NSP)	25.93	5	MS	29.63	5	MS
13	ICS-271-1	88.89	9	HS	88.89	9	HS
14	ICS-253	25.93	5	MS	25.93	5	MS
15	BCG-10	85.19	9	HS	77.78	9	HS
16	BCG-23	96.30	9	HS	92.59	9	HS
17	RG-2661 (GS)	66.67	9	HS	48.15	7	S
18	RG-2822	22.22	5	MS	25.93	5	MS
19	RG-3798 (OLD)	96.30	9	HS	96.30	9	HS
20	RG-2661-1	92.59	9	HS	100.00	9	HS
21	RG-2787	40.74	7	S	33.33	7	S
22	RG-109	74.07	9	HS	66.67	9	HS

(Statistical tool applied here is the descriptive statistics such as mean and standard deviation has been used to analyses)

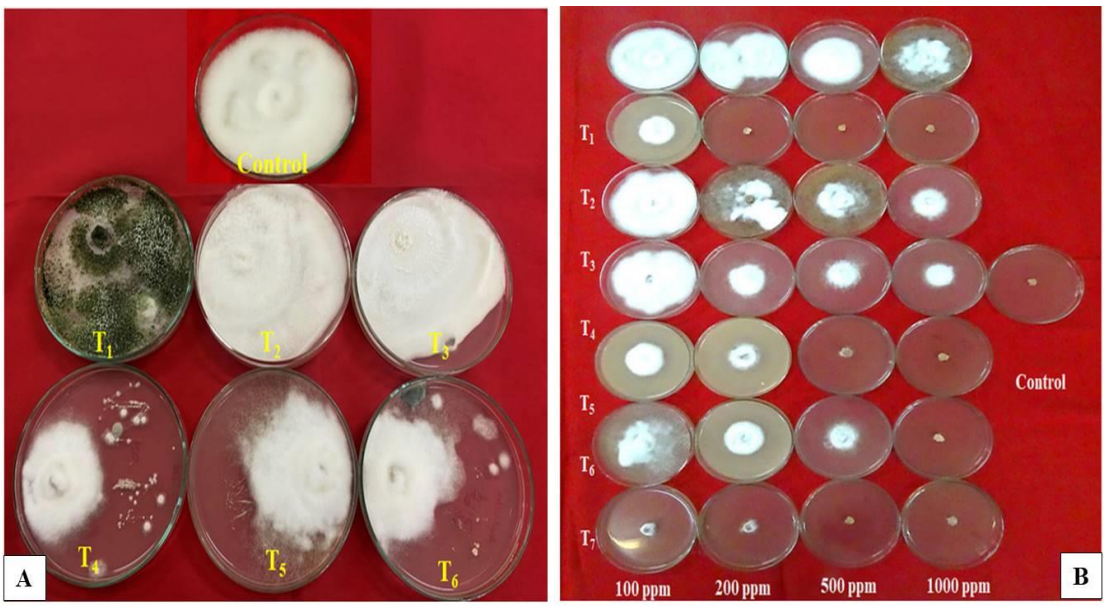

Fig. 3. A: *In vitro* evaluation of bio control agents against *B. ricini* infecting castor. B: *In vitro* evaluation of combination fungicides against *B. ricini* infecting castor

Germplasm lines namely HCG-26 (11.11 %), RG-72-1 (GS) (18.52 %) and TSIL- 3 (11.11 %) were moderately resistant to gray mold in detached spike method. In detached capsule method germplasm lines *viz*., HCG-26 (11.11 %) and RG-72-1 (GS) (18.52 %) were moderately resistant to *Botryotinia* gray mold. Nine germplasm lines namely ICS- 272 (25.93 %), TSIL-1 (25.93 %), TSIL-2 (29.63 %), ICS-234 (29.63 %), ICS-248 (25.93 %), ICS-253 (25.93 %), ICS-272(NSP) (25.93 %), RG-2822 (22.22 %) and TSIL-4 (22.22 %) were moderately susceptible to gray mold incidence in detached spike method. Ten germplasm lines *viz*., TSIL-2 (29.63 %), TSIL-3 (25.93 %), ICS-248 (22.22 %), ICS-253 (25.93 %), ICS-272 (29.63 %), TSIL-1 (22.22 %), ICS-234 (25.93 %), ICS-272(NSP) (29.63 %), RG-2822 (25.93 %) and TSIL-4 (22.22 %) were moderately susceptible to gray mold incidence in detached capsule method.

Thirteen germplasm lines namely MI-54 (40.74 %), RG-2787 (40.74 %), ICS-245 (33.33 %), ICS-258 (48.15 %), TSIL-8 (48.15 %), TSIL-17 (48.15 %), TSIL-18 (44.44 %), TSIL-20 (48.15 %), TSIL-21 (44.44 %), TSIL-22 (40.74 %), TSIL-24 (48.15 %), TSIL-25 (51.85 %) and TSIL-27 (48.15 %) were susceptible to gray mold incidence in detached spike method. Germplasm lines *viz*., MI-54 (37.04 %), RG-2661 (GS) (48.15 %1), RG-2787 (33.33 %), ICS-245 (37.04 %), ICS-258 (40.74 %), TSIL-8 (48.15 %), TSIL-17 (48.15 %), TSIL-18 (37.04 %), TSIL-20 (48.15 %), TSIL-24 (40.74 %), TSIL-25 (48.15 %) and TSIL-27 (44.44 %) were susceptible to *Botryotinia* gray mold in detached capsule method.

Nineteen germplasm lines namely MI-56 (100 %), MI-72 (96.30 %), MI-74 (92.59 %), ICS-270 (85.19 %), ICS-271-1 (88.89 %), BCG-10 (85.19 %), BCG-23 (96.30 %), RG-3798 (OLD) (96.30 %), RG-2661 (GS) (66.67 %), RG-2661-1 (92.59 %), RG-109 (74.07 %), TSIL-9 (77.78 %), TSIL-10 (74.07 %), TSIL-11 (62.96 %), TSIL-13 (59.26 %), TSIL-14 (55.56 %), TSIL-16 (92.59 %), TSIL-23 (74.07 %) and TSIL-26 (77.78 %) were highly susceptible to gray mold incidence in detached spike method. Twenty germplasm lines *viz*., MI-56 (92.59 %), MI-72 (100 %), MI-74 (96.30 %), ICS-270 (77.78 %), ICS-271-1 (88.89 %), BCG-10 (77.78 %), BCG-23 (92.59 %), RG-3798 (OLD) (96.30 %), RG-2661-1 (100 %), RG-109 (66.67 %), TSIL-9 (88.89 %), TSIL-10 (62.96 %), TSIL-11(66.67 %),TSIL-13 (51.85 %), TSIL-14 (55.56 %), TSIL-16 (96.30 %), TSIL-21 (100 %), TSIL-22 (96.30 %), TSIL-23 (81.48 %) and TSIL-26 (77.78 %) were highly susceptible to gray mold incidence in detached capsule method.

Per cent disease incidence and reaction of different germplasm lines that were assessed for resistance under artificial condition. Artificial screening is the best method for assessment of resistance against *Botryotinia* gray mold incidence than natural screening method (Table 4). In natural screening, germplasm lines may escape disease incidence due to various factors such as non availability of congenial environmental condition for disease development or due to late sowing of castor seeds.

3.5 Evaluation of Bio Agents and Combination Fungicides Against Gray Mold of Castor

3.5.1 *In vitro* evaluation of bio agents against *B. ricini*

The antagonistic effect of three fungal and three bacterial antagonists were assessed through dual culture technique. Among the three fungal antagonists assessed for their efficacy in inhibiting the *Botryotinia* mycelial growth, *Trichoderma viride* registered significantly maximum mycelial growth inhibition (86.11 %), followed by *T. harzianum* 41 (75.19 %) and 74.07 per cent mycelial inhibition reported from *T. harzianum* B2. Among all fungal bio agents tested for their efficacy *T. viride* was found to be most effective in inhibiting the *Botryotinia* gray mold and the other two were also found effective in inhibiting the mycelial growth ofgray mold.

Among the three bacterial bio agents evaluated, *Pseudomonas fluorescens* exhibited maximum mycelial growth inhibition (71.48 %) followed by *Bacillus velezensis* P42 (50 %) and least mycelial growth was recorded in *B. velezensis* A6 (45.37 %). The results are depicted in Table 5 and Fig. 3A.

3.5.2 *In vitro* evaluation of combination fungicides against *B. ricini*

Screening of combination fungicides was done under the laboratory condition by following poison food technique. Seven combi products were evaluated for their efficacy against *B. ricini*. The per cent inhibition of the growth at four different concentrations over control was computed and presented in Table 6 and Fig. 3B.

Table 4. Summary of screening castor germplasm against *B. ricini*

Disease score	Per cent infection	Diseasereaction	Germplasm lines		
			Natural condition	**Detached spike method**	**Detached capsule method**
0	0	Immuneresistant	HCG-26, RG-72-1 (GS), TSIL-2, TSIL-3	-	-
1	1-10	Resistant	ICS-248, ICS-272, ICS-253, TSIL-19	TSIL-19	TSIL-19
3	11-20	Moderatelyresistant	TSIL-1, ICS-234, ICS -272(NSP), RG-2822, TSIL-4	HCG-26, RG-72-1 (GS), TSIL-3	HCG-26, RG-72-1 (GS)
5	21-30	Moderately Susceptible	MI-54, RG-2661 (GS), RG-2787, TSIL-8	ICS-272, TSIL-1, TSIL-2, ICS-234, ICS-248, ICS-253, ICS-272(NSP), RG-2822, TSIL-4	TSIL-2, TSIL-3, ICS-248, ICS-253, ICS-272, TSIL-1, ICS-234, ICS-272(NSP), RG-2822,TSIL-4
7	31-50	Susceptible	TSIL-9, TSIL-10, TSIL-11, TSIL-13, TSIL-14, TSIL-16, TSIL-17, TSIL-18, TSIL-20, TSIL-21, TSIL-22, TSIL-23, TSIL-24, TSIL-25, TSIL-26, TSIL-27	MI-54, RG-2787, ICS-245,ICS-258, TSIL-8, TSIL-17, TSIL-18, TSIL-20, TSIL-21,TSIL-22, TSIL-24, TSIL-25, TSIL-27	MI-54, RG-2661 (GS), RG-2787, ICS-245, ICS-258, TSIL-8, TSIL-17, TSIL-18, TSIL-20, TSIL-24, TSIL-25, TSIL-27
9	>51	Highly susceptible	MI-56, MI-72, MI-74, ICS-245, ICS-258, ICS-270, ICS-271-1, BCG-10, BCG-23, RG-3798 (OLD), RG-2661-1, RG-109	MI-56, MI-72, MI-74, ICS-270, ICS-271-1, BCG-10, BCG-23,RG-3798 (OLD), RG-2661 (GS), RG-2661-1, RG-109, TSIL-9, TSIL-10, TSIL-11,TSIL-13, TSIL-14, TSIL-16, TSIL-23, TSIL-26	MI-56, MI-72, MI-74, ICS-270, ICS-271-1, BCG-10, BCG-23,RG-3798 (OLD), RG-2661-1, RG-109, TSIL-9, TSIL-10, TSIL-11,TSIL-13, TSIL-14, TSIL-16, TSIL-21, TSIL-22, TSIL-23, TSIL-26

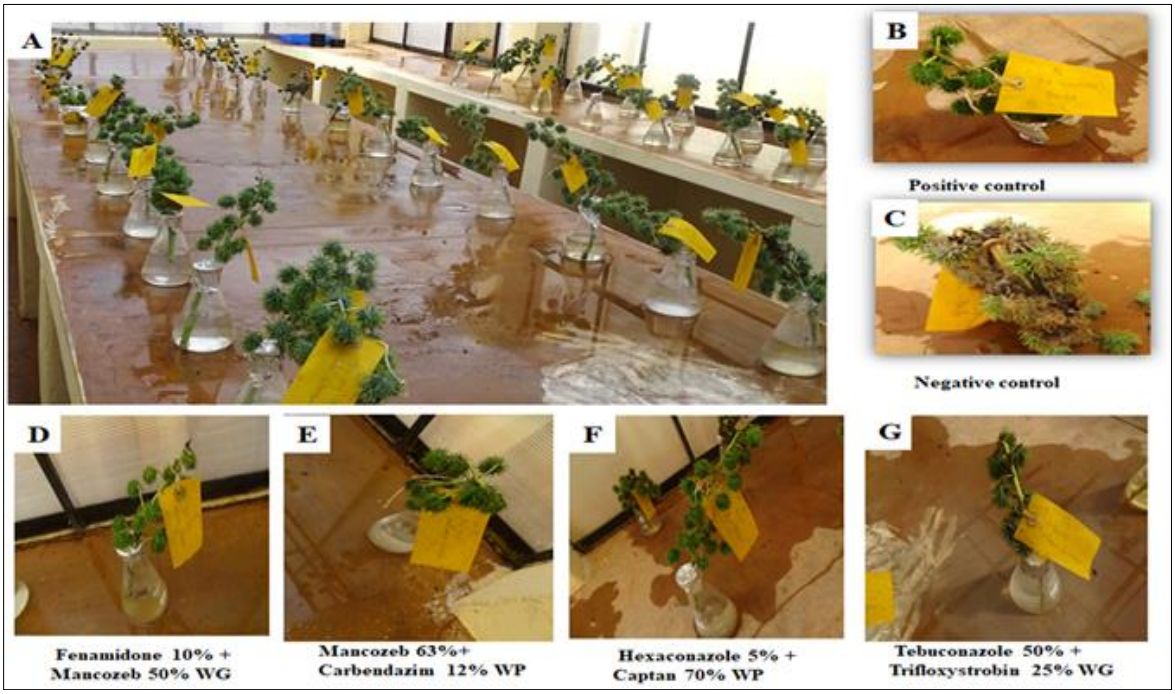

Fig. 4. *In vitro* evaluation of combination fungicides in inhibiting *B. ricini* on detached spikes of castor

A: Experimental set up; B: Positive Control: Propiconazole 25EC @1mL/L; C: Disease control D: Fenamidone 10% + Mancozeb 50% WG; E: Mancozeb 63%+ Carbendazim 12% WP; F: Hexaconazole 5% + Captan 70% WP; G: Tebuconazole 50% + Trifloxystrobin 25% WG;
T8: Positive Control: Propiconazole 25EC @1mL/L; T9: Disease control

Table 5. *In vitro* evaluation of bio control agents against *Botryotinia ricini* infectingcastor

Tr. No.	Bio control agents	Average colony diameter (mm)**	Per cent inhibition over control (%)*
T1	*Trichoderma viride*	12.50	86.11
T2	*Trichoderma harzianum (*Th41)	22.302	75.19
T3	*Trichoderma harzianum* (ThB2)	23.30	74.07
T4	*Pseudomonas fluorescens*	25.70	71.48
T5	*Bacillus velezensis* (A6)	49.20	45.37
T6	*Bacillus velezensis* (P42)	45.00	50.00
	Control	90.00	
	C.V. (%)	1.14	
	S.Em ±	0.44	
	C.D. (P= 0.01)	1.90	

*Note: **Mean of three replication.*
Figures in parenthesis are arcsine transformed values. (Statistical analysis was performed for average colony diameter)

Between the combi products analysed, maximum mean per cent of inhibition was observed in Fenamidone 10% + Mancozeb 50% WG (99 %) followed by Tebuconazole 50% + Trifloxystrobin 25% WG (92.56 %) and 91. 67 per cent inhibition was recorded in Mancozeb 63%+ Carbendazim 12% WP. Least per cent inhibition of mycelial growth was observed in Dimethomorph 9% + Mancozeb 60% WG with a mean of 26.55 per cent. Among the tested concentrations maximum mean per cent of inhibition was observed in 1000 ppm (83.07 %), significantly superior over other concentrations *i.e*., 500 ppm (68.81 %), 250 ppm (61.88 %) and 100 ppm (47.85 %).

Fenamidone 10% + Mancozeb 50% WG recorded 100 per cent inhibition of mycelial growth at concentration of 500 and 1000 ppm

and at a concentration of 100 and 250 ppm it recorded 98 per cent inhibition. Followed by Mancozeb 63%+ Carbendazim 12% WP which recorded 100 per cent inhibition at a concentration of 1000 and 500 ppm concentration. Tebuconazole 50% + Trifloxystrobin 25% WG recorded 100 per cent inhibition of mycelial growth at concentration of 1000 ppm and at a concentration of 500 ppm it recorded 98 per cent inhibition and at 1000 ppm Pyraclostrobin 5%+ Metiram 55% WG recorded cent per cent growth inhibition. *In vitro* evaluation of combination fungicides provides useful and preliminary information regarding efficacy of combi products against the pathogen within a shortest period of time. In the present investigation, seven combi-products were tested at four concentrations each (100, 250, 500 and 1000 ppm).

3.5.3 *In vitro* evaluation of combination fungicides in inhibiting *B. ricini* on detached spikes of castor

In order to test the efficacy of combi products in glasshouse studies, best performed combi products from *in vitro* studies were selected and evaluated them on detached spikes of castor in glass house at 500 and 1000 ppm concentration (Table 7 and Fig. 4).

Between the combi products evaluated maximum mean growth inhibition of mycelia was observed in Fenamidone 10% + Mancozeb 50% WG (96.30 %) which is superior over other treatment, followed by Mancozeb 63%+ Carbendazim 12% WP (81.48 %), Hexaconazole 5% + Captan 70% WP and Tebuconazole 50% + Trifloxystrobin 25% WG (81.48 %) followed by others. Least mycelial inhibition was noticed in Dimethomorph 9%+ Mancozeb 60% WG (25.92 %), however Propiconazole has showed complete mycelial inhibition (100 %). Among the tested concentration maximum mean per cent of inhibition was observed in 1000 ppm (71.19 %) followed by 500 ppm concentration (58.03 %).

Fenamidone 10% + Mancozeb 50% WG at both 1000 and 500 ppm concentration was found to be most effective in inhibiting the mycelial growth (96.30 %), followed by Mancozeb 63%+ Carbendazim 12% WP, Hexaconazole 5% + Captan 70% WP and Tebuconazole 50% + Trifloxystrobin 25% WG 88.89 per cent growth inhibition at a concentration of 1000 ppm. Least inhibition was observed in Dimethomorph 9% + Mancozeb 60% WG (7.41 %) at 500 ppm concentration. The study reveals that among the combi products tested Fenamidone 10% + Mancozeb 50% WG was to be the most effective in inhibiting the mycelial growth.

4. DISCUSSION

The research on *B. ricini* (Godfrey) Whetzel, a well-known necrotrophic pathogen that is thought to be extremely damaging and capable of causing a 100 per cent. reduction in seed production, has led to studies on castor grey mould. Castor germplasm lines were screened to determine how they responded to the disease in both natural and artificial environments. *In vitro* and glasshouse conditions, fungicides and biocontrol agents were used to create management techniques against *B. ricini*. The varied reaction of castor germplasm lines is attributed to the genetic constitution of each line and it suggests the availability of resistance in the germplasm lines screened. The screening of germplasm lines helps us to understand the magnitude of reaction of the lines to gray mold incidence. Those lines which exhibited absolute resistance can be exploited by the breeders in developing the varieties/hybrids which are free from the disease. These can be tested over locations in the endemic areas for hybrid development and commercial cultivation.

The above results are supported by Boyer et al., [30] who conducted a simple technique for screening 20 castor germplasm against gray mold disease. Among them germplasm lines like RG-1139, CI-1, CI-2 showed better resistant to the gray mold disease. Biological control proves to be a potential, eco-friendly and non-chemical method to control pathogens inciting various plant diseases with no hazardous effect on the health of other living beings. Therefore, it can play an important role in the integrated disease management system to control the gray mold of castor.

In the present investigation the *in vitro* evaluation of bio agents on inhibition of growth of *B. ricini* were carried out by dual culture technique. Among all the fungal bio agents used all three fungi were found to be effective in inhibiting *B. ricini* of castor, *T. viride* showed maximum per cent inhibition of mycelia (86.11 %) followed by *T. harzianum* 41 (75.19 %) and among the bacterial bio agents P. fluorescens was found to be effective in inhibiting the mycelial growth (71.48 %) compared to other two bacterial antagonists and these studies are supported with Simionato et al., [31] Islam et al., [32] Basha et al., [33].

Table 6. *In vitro* evaluation of combination fungicides against *Botryotinia ricini* infecting castor

Tr. No.	Combination fungicides	Per cent inhibition over control**				Mean
		Concentration (ppm)				
		100	250	500	1000	
T1	Dimethomorph 9% + Mancozeb 60% WG	15.50 (23.18)	20.33 (26.80)	27.78 (31.81)	42.59 (40.74)	26.55 (31.02)
T2	Mancozeb 63%+ Carbendazim 12% WP	66.67 (54.74)	96.67 (79.50)	100.00 (90.00)	100.00 (90.00)	91.67 (73.22)
T3	Iprovalicarb 5.5% +Propineb 61.25% WP	10.33 (18.75)	27.78 (31.81)	27.78 (31.81)	66.67 (54.74)	33.14 (35.15)
T4	Hexaconazole 5% + Captan 70% WP	27.78 (31.81)	42.59 (40.74)	42.59 (40.74)	72.22 (58.19)	46.30 (42.88)
T5	Tebuconazole 50% + Trifloxystrobin 25%WG	83.33 (65.91)	88.89 (70.53)	98.00 (81.87)	100.00 (90.00)	92.56 (74.17)
T6	Pyraclostrobin 5%+ Metiram 55% WG	33.33 (35.26)	55.56 (48.19)	85.50 (67.62)	100.00 (90.00)	68.60 (55.92)
T7	Fenamidone 10% + Mancozeb 50% WG	98.00 (81.87)	98.00 (81.87)	100.00 (90.00)	100.00 (90.00)	99.00 (84.26)
Mean		47.85 (43.77)	61.88 (51.87)	68.81 (56.05)	83.07 (65.70)	65.40 (53.97)
		S.Em ±		C.D. (P=0.01)		
Fungicide (F)		0.33		0.95		
Concentration (C)		0.25		0.71		
F×C		0.67		1.89		

*Note: **Mean of three replication.*
Figures in parenthesis are arcsine transformed values.

Most fungi have chitin and β (1-3) glucan as essential constituent in their cell wall. Mechanism for bio control by *Trichoderma* sp. is antibiosis, lysis, competition and mycoparasitism, their ability to suppress pathogens is mainly due to coiling and disintegration of hyphae of the pathogen.

The above results are in accordance with Xiangming et al., [34] who conducted an experiment to check the antagonistic effect of 12 isolates of *Trichoderma* against *Amphobotrys ricini* under laboratory and greenhouse conditions. Among the tested antagonists, *T. asperellum* 1, *T. harzianum* 5 and *T. asperellum* 3 recorded significantly maximum per cent inhibition of mycelial growth (77.96, 77.41 and 77.04 per cent, respectively). Hosen et al., [35] also reported the efficiency of *T. harzianum* strains in inhibition of mycelial growth, A comparable outcome has also been documented in Rashid et al., [36] and Rajeswari & Balasupramani, 2020 in different crops. The *in vitro* evaluation revealed that among the combi-products tested Fenamidone 10% + Mancozeb 50% WG and Mancozeb 63%+ Carbendazim 12% WP, were found to be the most effective in inhibiting the mycelial growth at 1000 and 500 ppm concentration and the least was Dimethomorph 9% + Mancozeb 60% WG at all concentrations. This may be due to inclusive effect of one systemic and another non-systemic fungicide which might have inhibited the mycelial growth.

Glass house evaluation of best performed combination fungicides at 500 and 1000 ppm concentration on detached spikes of castor revealed that maximum mycelial growth inhibition was recorded in Fenamidone 10% + Mancozeb 50% (96.30 %) at both concentration and it was statistically on par with Propiconazole 250EC and least mycelial growth inhibition was observed in Dimethomorph 9% + Mancozeb 60% at both concentration [36].

The results are in accordance with Rajeswari & Balasupramani, [37] Saikia et al., [38] who evaluated three combination fungicides [Acrobat MZ690 (0.2%); Secure 600 WG (0.2%) and Companion (0.2%)] with different modes of action on a *Botrytis* gray mold susceptible chickpea variety. The results revealed that companion (Mancozeb 63 % + carbendazim 12 %) was the most effective in recording the lowest disease severity and the maximum increase of grain yield in chickpea [39,40].

Table 7. *In vitro* evaluation of combination fungicides in inhibiting *B. ricini* on detached spikes of castor

Tr. No.	Combination fungicides	Per cent inhibition over control**		Mean
		Concentration (ppm)		
		500	1000	
T1	Dimethomorph 9% + Mancozeb 60% WG	7.41 (15.79)	44.44 (41.81)	25.92 (30.61)
T2	Mancozeb 63%+ Carbendazim 12% WP	74.08 (59.39)	88.89 (70.53)	81.48 (64.51)
T3	Iprovalicarb 5.5% +Propineb 61.25% WP	51.85 (46.06)	74.08 (59.39)	62.96 (52.51)
T4	Hexaconazole 5% + Captan 70% WP	74.08 (59.39)	88.89 (70.53)	81.48 (64.51)
T5	Tebuconazole 50% + Trifloxystrobin 25% WG	74.08 (59.39)	88.89 (70.53)	81.48 (64.51)
T6	Pyraclostrobin 5%+ Metiram 55% WG	44.44 (41.81)	59.26 (50.34)	51.85 (46.06)
T7	Fenamidone 10% + Mancozeb 50% WG	96.30 (78.91)	96.30 (78.91)	96.30 (78.91)
T8	Propiconazole 25EC @1mL/L (Positive control)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T9	Disease control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		58.03 (49.62)	71.19 (57.54)	64.64 53.49)
		S.Em ±	C.D. (P=0.05)	
Fungicide (F)		3.38	9.74	
Concentration (C)		1.59	4.59	
F×C		4.78	13.77	

*Note: **Mean of three replication.*
Figures in parenthesis are arcsine transformed values.

5. CONCLUSION

Out of 47 germplasm lines screened for resistance against *B. ricini*, TSIL-19 (7.41%) showed resistance to gray mold under both detached spike and capsule methods. Germplasm lines HCG-26, RG-72-1 (GS), and TSIL-3 were moderately resistant (11-20%). Trichoderma viride and Pseudomonas fluorescens exhibited the highest mycelial growth inhibition (86.11% and 71.48%, respectively). In vitro evaluation of seven combination fungicides revealed 100% mycelial growth inhibition by Fenamidone 10% + Mancozeb 50% WG and Mancozeb 63% + Carbendazim 12% WP at 500 and 1000 ppm, while Tebuconazole 50% + Trifloxystrobin 25% WG inhibited 100% at 1000 ppm. Dimethomorph 9% + Mancozeb 60% was less effective. Glasshouse evaluations showed Fenamidone 10% + Mancozeb 50% achieved 96.30% inhibition at both concentrations, comparable to Propiconazole 250EC, with Dimethomorph 9% + Mancozeb 60% being the least effective. Fresh insights into host resistance assessment in artificial conditions and evaluation of efficacy of combi fungicides on *B. ricini* will be beneficial for choosing the appropriate varieties/hybrids which are free from the disease and to manage the disease using the new generation fungicide molecules.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Option 1: The author(s) hereby declare that no generative AI technologies, such as Large Language Models (e.g., ChatGPT, COPILOT) or text-to-image generators, were used in the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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