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Eco-friendly management of brown spot disease of rice (*Oryza sativa* L.)

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Abstract
The four native isolates of different bio-agents *viz., Trichoderma viride, T. harzianum, Pseudomonas fluroscens* and *Bacillus subtilis* were evaluated against *Bipolaris oryzae* through dual culture technique. Among the bio agents *Trichoderma viride* produced maximum mycelial inhibition (73.08%) followed by *Trichoderma harzianum* (69.05%), *Pseudomonas fluroscens* (67.30%) and *Bacillus subtilis* (59.94%). The ten different botanicals extract tested by poisoned food technique at five per cent concentration. Among the botanicals leaves extract of turmeric produced maximum inhibition (67.04%) followed by rhizome extract of ginger (42.18%), bulb extract of garlic (31.48%), leaf extract of datura (24.33%), bulb extract of onion (22.22%), jetropha (21.85%), eucalyptus (20.37%) and neem (19.14%). Whereas, leaf extract of tulsi (9.63%) and leaf extract of karanj (4.81%) were found least effective in inhibiting the growth of the pathogen.

Keywords: Rice, antagonist, botanicals, *Bipolaris oryzae*

Introduction
Rice is the most important food crop of India and for the people living in the eastern and the southern parts of the country providing food to about half of the Indian population. The total area of the world under harvested rice is 167.24 million hectares producing 500 million tonnes of grains in 2020. In India rice is cultivated on 43.19 million hectares with an annual production of 117.47 million tonnes and annual yield 2550 kg/ha in 2019. Rice is infected by many diseases *viz.*, bacterial blight, leaf blast, sheath blight, false smut, grain smut, sheath rot, rice tungro etc. Brown spot of rice caused the major epidemic and contributed to the great Bengal famine of 1943. Brown spot infects on all leaf surfaces *viz.*, coleoptiles, leaves, leaf sheath, panicle branches, glumes and spikelet's which were appeared as minute spots on leaves, typical spots were brown in colour with grey or whitish centre resembling sesame seed with typical yellow halo over spots (Varalakshmi and Ladalakshami, 2018) [7]. Continuous use of pesticides and fungicides caused the resistance to many diseases and also causes the potential risk to human health and environment. Eco friendly management practices such as use of bio control agents and botanicals in controlling diseases proved to be effective in controlling many diseases and also considerably safe to environment and human health. Use of bio control agents and botanicals in controlling diseases proved to be effective in controlling many diseases and also considerably safe to environment and human health (Sajeena *et al*., 2019) [5].

Materials and Methods
Isolates of bio control agents were obtained from Dept. of Plant Pathology, N.M College of agriculture, Navsari and antagonistic activity of bio control agents *viz*., *Trichoderma viride* (Navsari isolate) *Trichoderma harzianum* (Navsari isolate), *Pseudomonas fluroscens* (Navsari isolate) and *Bacillus subtilis* (Navsari isolate) were studied for their efficacy against *B. oryzae* by dual culture technique (Dennis and Webster,1971) [2] and the experimental design is Completely Randomised Design (CRD) with three replications. Botanical extracts *viz.*, turmeric extract, rhizome extract of ginger, bulb extract of garlic, leaf extract of datura, bulb extract of onion, jetropha, eucalyptus, neem, leaf extract of tulsi, leaf extract of karanj of 5 per cent concentration was prepared and efficacy of botanicals were studied by using poisoned food technique under *in vitro* condition (Jatoi *et al*., 2015) and the experimental design is completely randomised design with three repetitions for each treatment.

Experiment under *in vitro*

Dual culture technique for the efficacy of antagonists against *B. oryzae*:

Different antagonists were evaluated for their antagonistic activity against *B. oryzae in vitro* by dual culture technique. The test organism and pathogen were grown separately on PDA medium. Seven days old culture, 5 mm mycelial disc of the test organism and pathogen was cut aseptically and placed opposite to each other approximately 60 mm apart from each other on to Petri plate containing 20 ml PDA. While, in bacterial antagonists the bacterial colony was streaked at one end of each Petri plate poured aseptically with 20 ml PDA/NA medium 24 hours prior to the pathogen inoculation. The plate with only pathogen (*B. oryzae*) was served as control. The plates were incubated at 27±2°C temperature and after seven days, the radial growth was measured. Inhibition zone was measured at 24 hours interval till the colony in the control plate covered with mycelium of pathogen. The per cent growth inhibition (PGI) was calculated by using the formula suggested by Vincent (1947) [8].

$$\text{Per cent growth inhibition} = \frac{\text{Radial growth in control (mm)} - \text{Radial growth in treatment (mm)}}{\text{Radial growth in control (mm)}} \times 100$$

Where

PGI= Per cent growth inhibition

DC= Average diameter of mycelial colony of control set (mm)

DT= Average diameter of mycelial colony of treated set (mm)

Poisoined food technique for the efficacy of botanicals against *B. oryzae*

Fresh and healthy plant parts *viz*., leaves, bulb, clove, rhizome of were collected and 50 g plant parts of each plant species washed thoroughly in tap water and finely crushed using grinder and then mixed with 50 ml sterilized distilled water. Each phyto-extracts thus obtained were filtered through double layered sterilized muslin cloth into 150 ml conical flasks and were plugged with non absorbant cotton. Thus filtered phyto-extracts autoclaved at 15 psi for 20 minutes before using these phyto-extracts in poisoned food technique. Autoclaved extracts were individually added in previously sterilised PDA 5 per cent extracts were mixed thoroughly at the time of pouring in the previously sterilized Petri plates. The Petri plates containing PDA were inoculated aseptically after solidification with 5 mm mycelial disc from 7 days old culture of *B. oryzae* with the help of sterilized cork borer. Three repetitions of each treatment were maintained and the plates without phyto-extracts served as control. The plates were incubated at 27±2 °C temperature in an incubator for seven days.

Results and Discussion

Effect of bio agents on growth of *B. oryzae*

The results presented in (Table-1) (Fig-1) revealed that all the antagonists screened were significantly superior in their efficacy over the control and per cent inhibition of the mycelial growth of the pathogen was calculated. Among different bio-control agents tested against *B. oryzae*, the treatment of *T. viride* was most effective and showed maximum (73.08%) growth inhibition of mycelial growth of the pathogen and appeared to be the most superior in its efficacy over all the antagonists tested which was statistically at par with *T. harzianum*, figures in parantheses are original values, figures outside parantheses are arc sin transformed values. (69.05%) and next best in order of merit was *P. flourescens* (67.30%). The least effective was *B. subtilis* (59.94%) growth inhibition, were exhibited decreasing trend in per cent growth inhibition of *B. oryzae*. The present results are in harmony with the findings of earlier workers, *viz*., Sarkar *et al*. (2014) reported that the isolate of *T. viride* (TV_5) showed maximum per cent inhibition (99.20%) where antagonist completely over grew the pathogen, and the isolate of *T. harzianum* (TH_2) showed minimum per cent inhibition (72.22%) of the brown spot pathogen. Balabaskar *et al*. (2016) [1] observed that among all bacterial antagonists tested, bacterial antagonist (*P. fluorescens*) was most efficient in controlling brown spot pathogen.

Effect of botanicals against the pathogen *in vitro*

The results presented in (Table-2) and depicted in Fig.-2 revealed that all the plant extracts inhibited the growth of the fungus significantly as compared to control. Among the effective botanicals, turmeric extract produced maximum inhibition (67.04%) which was found significantly superior over rest of the treatments. Next best botanical in order of merit were rhizome extract of ginger (42.18%), bulb extract of garlic (31.48%), leaf extract of datura (24.33%), bulb extract of onion (22.22%), jetropha (21.85%), eucalyptus (20.37%) and neem (19.14%). Whereas, leaf extract of tulsi (9.63%) and leaf extract of karanj (4.81%) were found least effective in inhibiting the growth of the pathogen. The results obtained in this experiment are close with the findings of earlier workers, Dorneles *et al*. (2018) [3] who reported highest per cent reduction of sporulation of *B. oryzae* by turmeric extract at different concentrations. Jatoi *et al*. (2015) reported lowest linear colony growth of fungi (*B. oryzae*) with ginger, followed by garlic and datura at 5, 10, 15, 100 ml/medium doses.

Table 1: Effect of bio agents against pathogen under *in vitro* condition

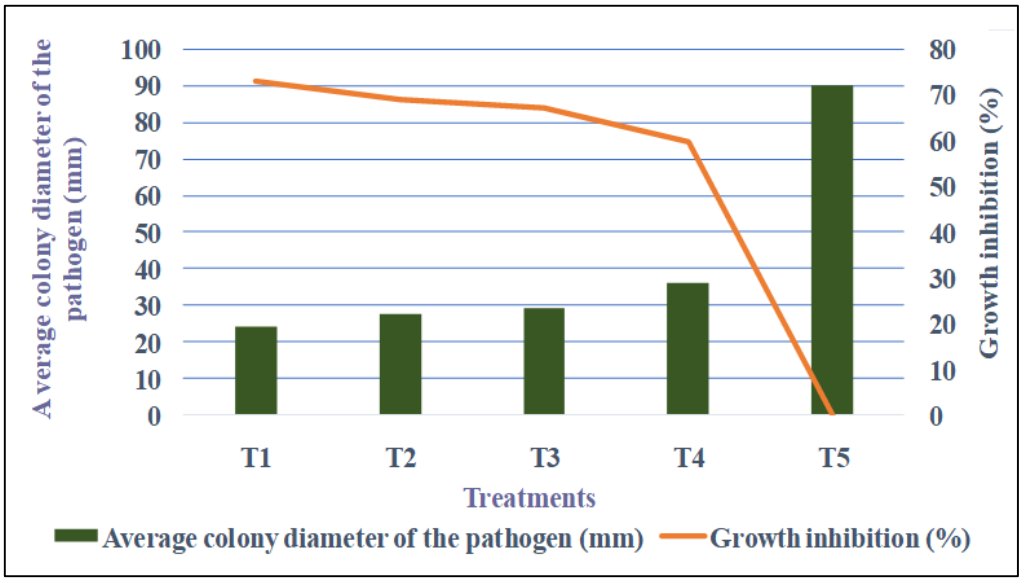
Tr. No.	Name of antagonists	Average colony diameter of pathogen (mm)	Growth inhibition (%)
T_1	*Trichoderma viride* (Navsari isolate)	24.23	58.75(73.08)
T_2	*Trichoderma harzianum* (Navsari isolate)	27.85	56.20(69.05)
T_3	*Pseudomonas fluorescens* (Navsari isolate)	29.42	55.13(67.30)
T_4	*Bacillus subtilis* (Navsari isolate)	36.05	50.73(59.94)
T_5	Control (Untreated)	90.00	0 (0.00)
S.Em (±)			0.65
C.D ($P \leq 0.01$)			2.72
C.V (%)			2.07

Figures in parantheses are original values, Figures outside parantheses are arc sin transformed values.

Table 2: Effect of botanicals against pathogen under *in vitro* condition

Treat. No.	Common name	Botanical name	Plant parts for extracts	Conc. (%)	Average diameter of the pathogen (mm)	Growth inhibition (%)
T_1	Onion	*Allium cepa* L.	Bulb	5%	70.00	28.12(22.22)
T_2	Neem	*Azadirachta indica* L.	Leaves	5%	72.77	25.94 (19.14)
T_3	Tulsi	*Ocimum sanctum* L.	Leaves	5%	81.33	18.08 (9.63)
T_4	Garlic	*Allium sativum* L.	Cloves	5%	61.67	34.11 (31.48)
T_5	Ginger	*Zingiber officinalis* Rosa	Rhizome	5%	52.03	40.50 (42.18)
T_6	Eucalyptus	*Eucalyptus citriodora* Hook	Leaves	5%	71.67	26.82 (20.37)
T_7	Karanj	*Pongamiaglubra* L.	Leaves	5%	85.67	12.67 (4.81)
T_8	Datura	*Datura stamoneum* L.	Leaves	5%	68.10	29.56 (24.33)
T_9	Jetropha	*Jetrophacurcas* L.	Leaves	5%	70.37	27.86 (21.85)
T_{10}	Turmeric	*Curcuma longa* L.	Rhizome	5%	29.67	54.97 (67.04)
T_{11}	Control	-	-	-	90.00	0.00 (0.00)
S.Em (±)						0.60
C.D ($P \leq 0.01$)					-	2.34
C.V (%)					-	2.17

Figures in parantheses are original values, Figures outside parantheses are arc sin transformed values.



T_1 - *Trichoderma viride*, T_2 - *Trichoderma harzianum*, T_3 - *Pseudomonas fluorescens*, T_4 – *Bacillus subtilis*, T_5 - control

Fig 1: Effect of antagonists against the pathogen *in vitro*

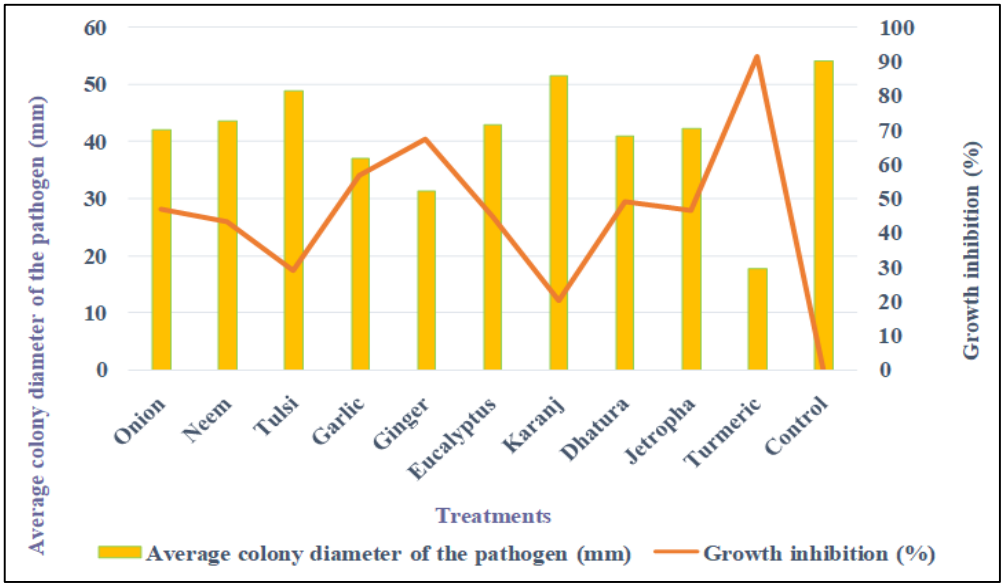


Fig 2: Effect of botanicals against the pathogen *in vitro*

Conclusion

Four different bio agents were evaluated *in vitro* for their antagonistic activity against *B. oryzae* by dual culture method. Among them, *T. viride* was most effective and showed

maximum (73.08%) growth inhibition of mycelial growth of the pathogen and appeared to be the most superior in its efficacy over all the antagonists tested which was statistically at par with *T. harzianum* (69.05%). While, *B. subtilis* (59.94%) was exhibited decreasing trend in per cent growth inhibition. Ten different botanical extracts were evaluated for their inhibitory effect on mycelial growth of *B. oryzae* at 5 per cent concentration by poisoned food technique. Among them, turmeric extract showed maximum inhibition (67.04%) which was found significantly superior over rest of the treatments. Whereas, leaf extract of tulsi (9.63%) and leaf extract of karanj (4.81%) was found to be least effective in inhibiting the growth of the pathogen.

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