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Efficacy of bioagents and fungicides against banded leaf and sheath blight of maize caused by *Rhizoctonia solani f. sp. sasakii* Kuhn

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Abstract
Maize (*Zea mays* L.) is one of the most important cereal crop in the world. It is grown throughout the temperate, tropical and sub tropical zones and has worldwide significance as human food, feed for live stock and for industrial and pharmaceutical sectors as every part of maize plant has one or the other economic values. Banded leaf and sheath blight (BL&SB) caused by *Rhizoctonia solani f. sp. sasakii kuhn* Kühn is one of the important disease of maize that became serious in recent years and recognized as a major constraint for limiting production. This pathogen causes losses in grain yield to the extent of over 90 percent. Pantnagar has been recognized as a hot spot for banded leaf and sheath blight disease at national level. Present study was undertaken to see the *in vitro* effect of fungicides and bioagents against the pathogen. *In vitro*, Efficacy of fungicides against test pathogen was studied by poisoned food technique. The fungicide viz, propiconazole and carbendazim were evaluated at 5 different concentrations viz 1, 5, 10, 20 and 50 µg/ml where as mancozeb at 20, 50, 100, 200 and 300 µg/ml. The Efficacy of three bioagents viz *Trichoderma harzianum, Pseudomonas fluorescens and Trichoderma harzianum + Pseudomonas fluorescens* was tested using dual culture technique against test pathogen. Fungicides Propiconazole and Carbendazim @ 20 µg/ml completely inhibited the radial growth of test fungus where as in case of Mancozeb it was achieved @ 100 µg/ml. Among the bioagents maximum percent inhibition in mycelial growth of test pathogen was recorded in *Trichoderma harzianum + Pseudomonas fluorescens* (62.35%), followed by *Trichoderma harzianum* (44.41%) while least percent inhibition was recorded in *Pseudomonas fluorescens* (41.18%).

Keywords: *Rhizoctonia solani f. sp. sasakii* Kühn, fungicides, bioagents, maize

Introduction
Maize (*Zea mays* L.) is one of the most important cereal crop in the world. It is grown throughout the temperate, tropical and sub tropical zones and has worldwide significance as human food, feed for live stock and for industrial and pharmaceutical sectors as every part of maize plant has one or the other economic values. Banded leaf and sheath blight (BL&SB) caused by *Rhizoctonia solani f. sp. sasakii* Kühn is one of the important disease of maize that became serious in recent years and recognized as a maize constraint for limiting production. This pathogen causes losses in grain yield to the extent of over 90 percent. A number of fungicides have been found to be effective against *Rhizoctonia solani f. sp. sasakii* both under natural and artificial conditions (Roy and Saikia, 1976) [13]. Sinha (1992) [15] reported most effective control of Banded Leaf and Sheath Blight disease in maize with Bavistin 50 WP (carbendazim) resulting 87% disease control. Two foliar sprays of propiconazole 25% EC (Tilt), first at initial stages (30th or 40th days of planting) and second spray after ten days was effective in protecting the crop from the disease. Kumar and Jha (1999) [2] evaluated effectiveness of fungicidal spray of nine fungicides on the severity of Banded Leaf and Sheath Blight and grain yield of maize. Spraying of Bavistin (0.1%) resulted in minimum disease severity and maximum grain yield. He also reported that foliar sprays of propiconazole resulted in reduced disease severity followed by carbendazim, contaf, saaf and mancozeb. Dumitras (1984) [1] reported that strains of *Trichoderma viride* were highly antagonistic to *Rhizoctonia solani* and the effectiveness can be increased by 20% by combining biocontrol agents with fungicidal treatment. Significant control in sheath blight incidence and severity was obtained when the soil was amended with *T. viride*. Mehra (2008) [7] reported that the seed and soil treatment by *Trichoderma harzianum*-43 exhibited minimum disease severity followed by seed and soil treatment with *Trichoderma harzianum*-43 + *Pseudomonas fluorescens*-27. It also increased the yield, thousand grain weight. He also reported that all the application methods of biocontrol agents significantly increased the grain yield/ha when

compared with untreated plots, highest grain yield obtained from combination of *Trichoderma harzianum*-43 + *Pseudomonas fluorescens*-27 (20.2%) over the check. Joshi (2003) [6] reported that the antagonist *P. fluorescens* when tested for its efficacy against *R solani in vitro i*n dual culture, inhibition of the fungus was clearly observed around the antagonist, beyond which no growth of fungus was observed. Mishra (1998) [9] reported that integration of biocontrol agent and fungicide propiconazole 25% EC was more effective in reducing sheath blight of rice and increasing grain yield than integration of two biocontrol agents. Combination of *P. fluorescens* and propiconazole 25% EC gave best results with least severity. Other combination of biocontrol agents and fungicides viz., *Gliocladium virens* + propiconazole 25% EC and *Trichoderma virens* + propiconazole 25% EC were next in the order of efficacy. Rijal (2003) [12] reported that foliar spray of tilt @ 0.10% and bioagents @ 10 g/litre water through reduced the incidence of BL&SB but spray of Tilt was distinctly superior to sprays of bioagents.

Materials and Methods

The pathogen of banded leaf and sheath blight of maize was isolated from sclerotia collected from diseased plants of previous year from Crop Research Centre of the University, on potato dextrose agar (PDA) medium. The culture was purified and was maintained in laboratory for the use throughout the investigation.

Fungi derive their food from the substrate on which they grow. No universal medium is available on which all fungi could be grown satisfactorily. Therefore, it is necessary to find out a suitable medium which could support growth and sporulation of the organism. Thus to support the fungal growth and sporulation, different solid media were tested. Different media were used for purification, isolation and bioassay. The composition of medium is given below:

1 Potato Dextrose Agar

Popato (peeled and sliced)	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

2 Oat Meal Agar medium

Oat Meal	:	40 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

3 Corn Meal Agar medium

Corn meal	:	20 g
Peptone	:	20 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

4 Czapek's Dox medium

Sodium nitrate	:	2.0 g
Di potassium hydrogen orthophosphate	:	1.0 g
Magnesium sulphate	:	0.5 g
Ferrous sulphate	:	0.5 g
Sucrose	:	30.0 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

5 Richard's Agar medium

Potassium nitrate	:	10 g
Potassium dihydrogen orthophosphate	:	5.0 g
Magnesium sulphate	:	2.5 g
Ferric chloride	:	0.02 g
Sucrose	:	50.0 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

The ingredients of each medium were dissolved in 1000 ml of distilled water and boiled for 15 minutes and final volume was made to 1 liter by adding distilled water. Then media were transferred in 250 ml flasks and plugged with non absorbent cotton. Then it was sterilized in an autoclave at a temperature of 121.6 ºC for 15 minutes.

Selection of best media suited for growth of the test pathogen

Sterilized mlton media having temperature around 40 ºC was poured aseptically in sterilized Petri plates. After solidification these plates were centrally inoculated with 5 mm disc of 3 days old culture of test fungus. Five replications were maintained and incubated at 25 ± 2 ^{0}C. Radial growth of test fungus was measured up to 72 h of inoculation. Amongst the media tested for the growth.

***In vitro* screening of fungicides against the test pathogen**

In vitro, efficacy of different chemicals against test pathogen was studied by poisoned food technique (Grover and Moore., 1961) [5]. The chemicals viz., Propiconazole (Tilt-25% EC), Carbendazim (Bavistin-50% WP) and mancozeb (Dithene M-45 75% WP) were evaluated.

The concentration of each systemic fungicide was 1, 5, 10, 20, 25, µg/ ml. while mancozeb was 20,50, 100, 200 and 300 µg/ ml. Stock solution of fungicides was prepared on formulation in sterilized distilled water by dissolving weighed quantity of double concentrated fungicide in measured volume of sterilized distilled water.

This stock solution was used to prepare desired fungicidal solution by adding required amount of sterilized distilled water to stock solution using following formula.

$C_1V_1=C_2V_2$

Where,

C_1	=	Concentration of stock solution
V_1	=	Volume of stock solution to be added
C_2	=	Desired concentration
V_2	=	Volume of solution of desired concentration

The composition of double strength PDA was as following:

Potato	:	400 g
Dextrose	:	40 g
Agar-agar	:	40 g
Distilled water	:	1000 ml

The medium was prepared and sterilized at 15 psi pressure for 20 minutes and the fungicidal solution of desired concentration was added to the medium in 1:1 ratio (50 ml PDA+50 ml fungicidal solution). Twenty ml of fungicide amended PDA was poured in each sterilized Petri plate. After solidification of medium, each Petri plate was centrally inoculated with 5 mm mycelial disc cut from the edge of 3 day old culture of *Rhizoctonia solani* using sterilized cork borer. Five replications of each treatment were maintained. In check 50 ml sterilized distilled water added to 50 ml double strength PDA medium and inoculation was done.

Per cent inhibition of pathogen by fungicides

All the Petri plates were incubated at 25 ± 2 ºC. Colony diameter was measured at 24, 36, 48, 60 and 72 h after inoculation by measuring scale at right angle and mean colony diameter was calculated. Actual colony diameter was calculated by deducting the diameter of mycelial disc from mean colony diameter. Per cent inhibition of pathogen by fungicide was calculated using following formula.

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

Where,

I = Per cent inhibition

C = Colony diameter in check (mm)

T = Colony diameter in treatment (mm)

***In vitro* interaction between bioagents with test pathogen**

***In vitro* evaluation of fungal antagonist against test pathogen**

A dual culture technique (Mortan and Stroube., 1955) [10] was used for testing the antagonism between *Trichoderma harzianum* and test pathogen. After solidification of medium, mycelial discs were cut from three days old culture of test pathogen. With the help of 5 mm cork borer one disc was placed in each Petri plate from three day old culture of *Trichoderma harzianum.* One mycelial disc was kept in each plate opposite to mycelial disc of test pathogen. The distance between mycelial disc of *Trichoderma harzianum* and test pathogen was maintained 6 cm approx. The plates were incubated at 25 ± 2 ºC. Observations were taken at regular intervals starting after 12 h of inoculation to measure the radial growth of test pathogen and the diameter of inhibition zone in treated plates.

***In vitro* interaction of bacterial antagonist *Pseudomonas fluorescens* against test pathogen**

A dual culture technique was used for testing the antagonism between *Pseudomonas fluorescens* and test pathogen as recommended by Morton and Strobe (1955) [10]. The medium which was used to study antagonistic potential of *Pseudomonas fluorescens* was prepared by mixing equal amount of sterilized PDA and sterilized King's B medium. Twenty ml medium was poured in each sterilized Petri plates aseptically. After solidification mycelial discs were cut from three days old culture of test pathogen with the help of 5 mm diameter cork borer and one disc was placed in each Petri plate. Filter discs were cut with 5 mm diameter and autoclaved twice. These sterilized filter discs were soaked in 48 h old *Pseudomonas fluorescens* liquid culture in King's B broth. These paper discs were then air dried inside the laminar flow and one disc was kept in each plate opposite to mycelial disc of test pathogen. The distance between paper disc of *Pseudomonas fluorescens* and mycelial disc of test pathogen was maintained 6 cm approximately.

***In vitro* interaction of combination of fungal and bacterial antagonist against test pathogen**

A dual culture technique was used for testing the antagonism between *Pseudomonas fluorescens* and test pathogen *as* recommended by Mortan and Stroube (1955) [10]. The medium which was used to study antagonistic potential of *Trichoderma harzianum* + *Pseudomonas fluorescens* was prepared by mixing equal amount of sterilized PDA and sterilized King's B medium. The 20 ml medium was poured into each sterilized Petri plates aseptically. After solidification of medium, mycelial discs were cut from three days old culture of test pathogen with the help of 5 mm diameter cork borer and one disc was placed in each Petri plate. Filter discs were autoclaved twice. These sterilized filter discs were soaked in 48 h old *Pseudomonas fluorescens* liquid culture in King's B broth. These paper discs were then air dried inside the laminar flow and one disc was kept in each plate opposite to mycelial disc of test pathogen. The distance between paper disc of *Pseudomonas fluorescens*, mycelial disc of *Trichoderma harzianum* and mycelial disc of test pathogen was maintained 3 cm approximately. The plates were incubated at 25 ± 2 ºC. Observations were recorded at regular interval starting after 12h of inoculation to measure the radial growth of test pathogen and the diameter of inhibition zone. A check was also maintained.

Results and Discussions

The test fungus was isolated from diseased leaf sheath of maize plants. The pathogen produced white colonies on PDA medium. In later stages, it produced pale brown to black colored sclerotia. Sclerotia vary in shape and size and sometimes they may be aggregated or joined together.

Effect of different media on growth of the test pathogen

In order to find out a suitable medium that could support good growth of the test pathogen semi natural (Oat Meal Agar, Corn Meal Agar and Potato Dextrose Agar) and synthetic media (Richard's Agar and Czapeck's Dox Agar) were tested.

Result revealed that all the media supported fungal growth (Table 1, Plate 1) but Potato Dextrose Agar medium supported best growth (85.0 mm) after 72 h, which was statistically significant as compared to other media tried in the present investigation while minimum growth (12.0 mm) was recorded in Richards Agar medium. Potato Dextrose Agar medium supported best growth of test pathogen, hence Potato Dextrose Agar medium was used for further studies.

***In vitro* evaluation of chemicals against the test pathogen**

Evaluation data of fungicides on radial growth of test pathogen (Table 2, Plate 2) indicated that increase in per cent inhibition was invariably proportional to increase in concentration of the test fungicides. Among the three fungicides evaluated propiconazole @ 20 µg/ml and carbendazim @ 20 µg/ml were found best and significantly at par as compared to mancozeb which completely arrested the growth @100 µg/ml. Rijal (2003) [12] reported that tilt used at three concentrations (5, 10 and 50 µg/ml) suppressed the growth of *Rhizoctonia solani*. Goswami (2008) [4] also observed bavistin most effective in inhibiting the growth of the *R. solani* followed by contaf and tilt under the systemic category while, mancozeb and saaf under the non systemic category resulted in complete inhibition of the pathogen at all the concentrations.

***In vitro* interaction between bioagents with test pathogen alone and in combination**

The interaction between test pathogen and fungal antagonist *Trichoderma harzianum* and with antagonistic bacteria *P. fluorescens* was evaluated by dual culture technique. Clear inhibition zone was formed around the pathogen. In inhibition zone, no mycelial growth of test pathogen was visible. Maximum per cent inhibition in mycelial growth of test pathogen was recorded in *Trichoderma harzianum* + *P.*

fluorescens (62.35%), followed by *Trichoderma harzianum* alone (44.41%) and *P. fluorescens* (41.18%) which were significantly inhibited the mycelial growth in comparision to check (Table 3, Plate 3). Sharma (1996) [16] found *Trichoderma harzianum, Trichoderma viride, Gliocladium virens, and Trichoderma sp* highly effective in inhibiting the growth of *R. solani* within 10 days of inoculation whereas none of the fluorescent Pseudomonas bacteria could inhibit the growth of *R.solani.* Singh (2000) [14] have reported effectiveness of *Trichoderma harzianum, Gliocladium virens, Aspergillus sp., Neurospora crassa* and *Penicillium sp.* in inhibiting the mycelial growth, suppression of sclerotial formation and germination of sclerotia of *R solani.* Fungal bioagents, and some bacterial bioagents especially *Gliocladium virens, Pseudomonas fluorescens* have been found to be effective in controlling sheath blight caused by *R. solani***.** (Gangopadhyay and Chakrabarti, 1982; Mew and Rosales, 1984; Ou, 1985; Sharma, 1996) [3, 8, 11, 16]

Fig 1.1: Oat Meal Agar

Fig 1.2: Potato Dextrose Agar

Fig 1.3: Czapek's Dox

Fig 1.4: Corn Meal Agar

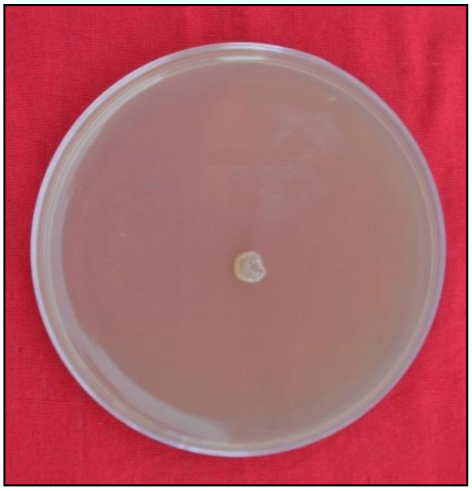

Fig 1.5: Richard's Agar

Plate I: Growth of *Rhizoctonia solani* on different medium

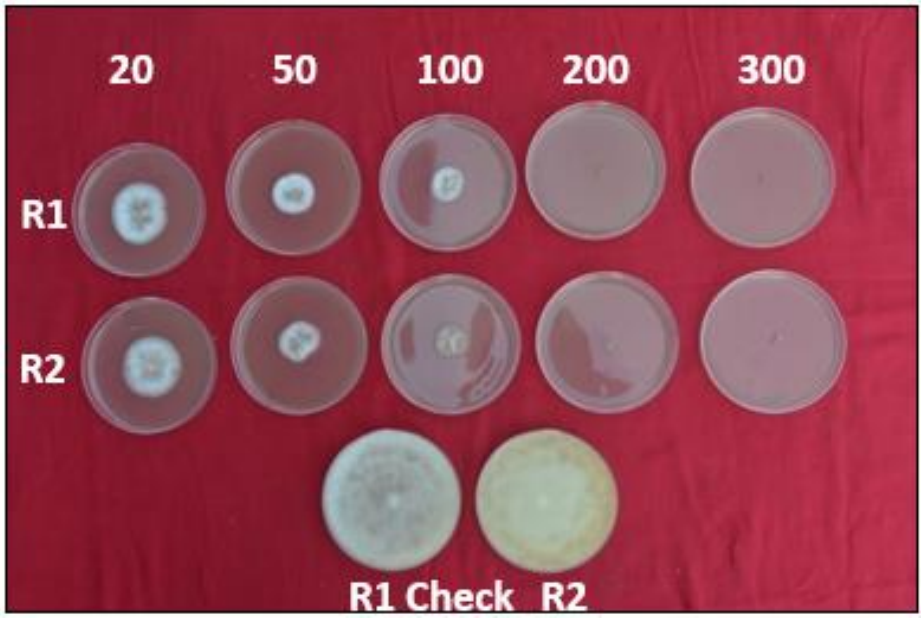

Fig 2.1: Bioassay of Mancozeb

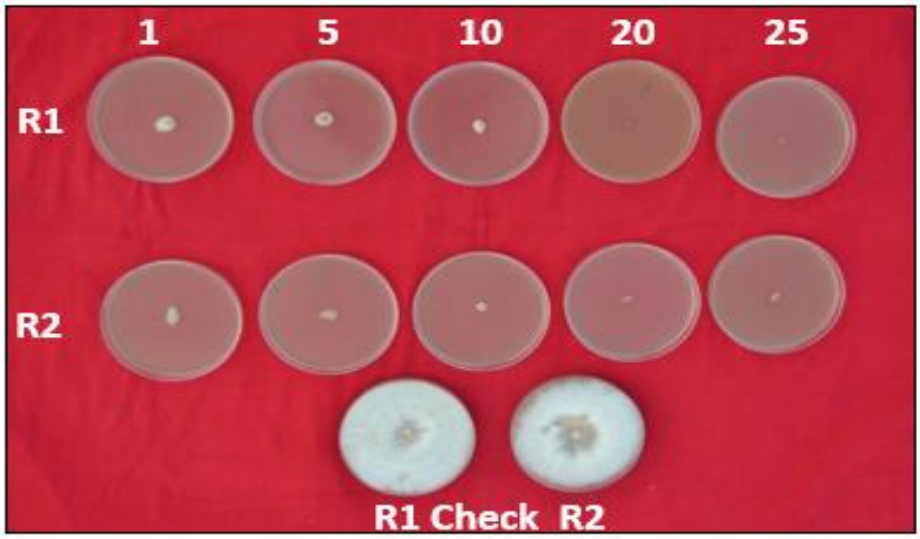

Fig 2.2: Bioassay of Carbendazim

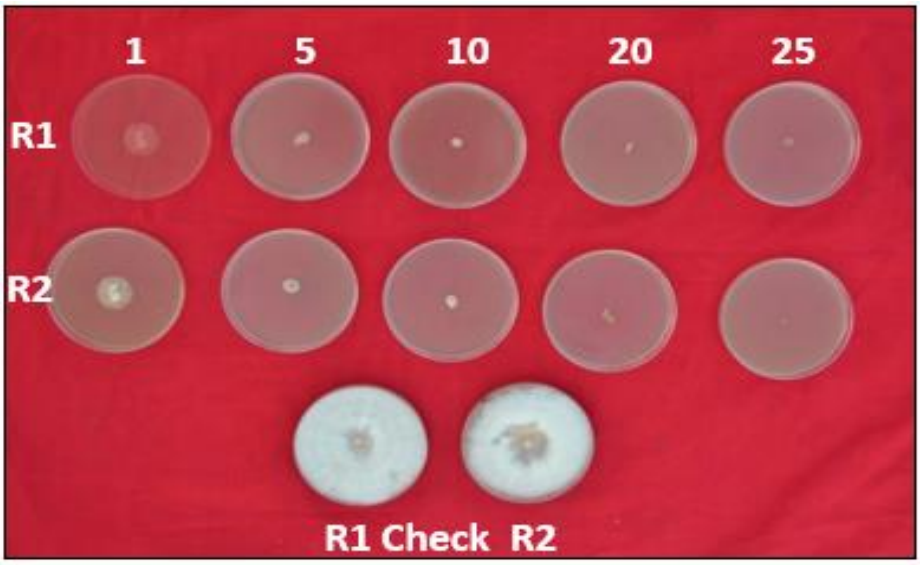

Fig 2.3: Bioassay of Propiconazole

Plate II

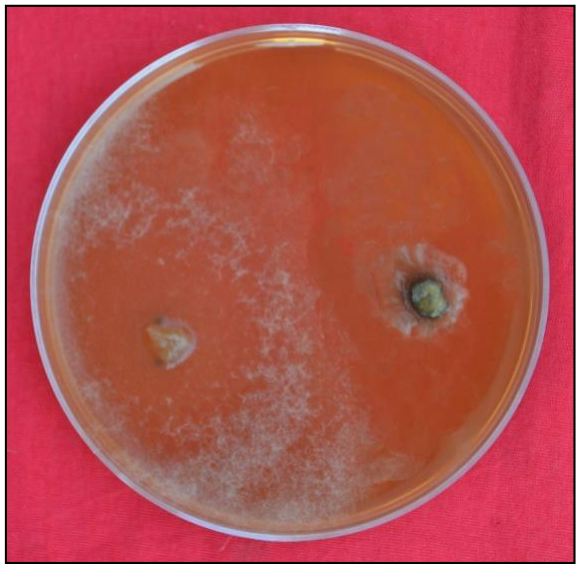

Fig 3.1: Interaction between *R. solani* and *T. harzianum*

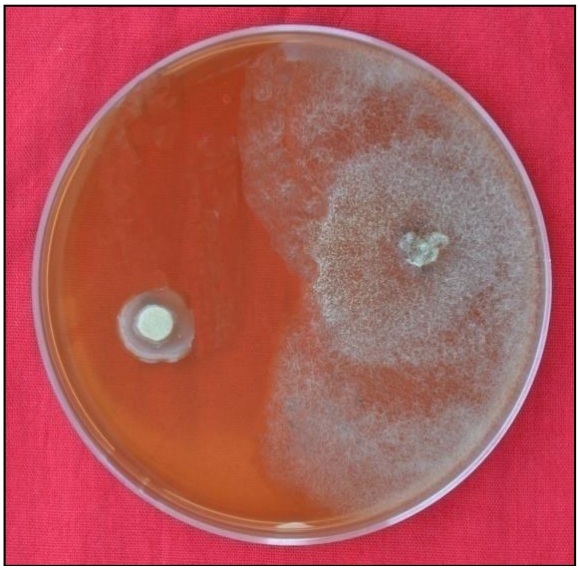

Fig 3.2: Interaction between *R. solani* and *P. fluorencens*

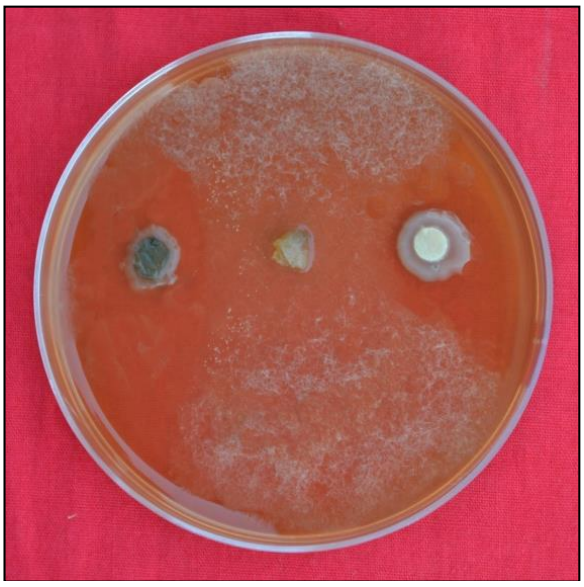

Fig 3.3: Interaction between *R. solani*, *T. harzianum* and *P. fluorencens*

Fig 3.4: Growth of *R. solani* on check

Plate III

Conclusion

Potato Dextrose Agar medium supported best growth of the test fungus. Propiconazole and carbendazim were found equally effective in completely inhibiting the radial growth of R. solani f. sp. sasakii under *in vitro* @ 20 µg/ml. A combination of *T. harzianum +P. fluorescens* was more affective compared to singly use of *T. harzianum or P. fluorescens*.

Acknowledgement

Support provided by Directorate of Research of GBPUAT, Pantnagar and AICRIP on Maize for conducting the field experiment is duly acknowledged.

Table 1: Effect of different media on growth of test pathogen at 25 ± 2 ºC after 72h of incubation

Sl. No.	Solid media	Colony diameter (mm)	Actual colony diameter (mm)	Percent radial growth
1	Oat Meal Agar medium	25.8	20.8	24.47
2	Richard's Agar medium	17.0	12.0	14.12
3	Corn Meal Agar medium	23.8	18.8	22.12
4	Czapek's Dox medium	31.6	26.6	31.29
5	Potato Dextrose Agar	90.0	85.0	100.00
CD at 5%				1.637
SEM ±				0.555

Table 2: Effect of different fungicides on the growth of test pathogen at 25 ± 2 ºC after 72 h of incubation

Treatment	Fungicides	Concentration (µg/ml)	Radial growth (mm)	Actual radial growth (mm)	% inhibition in radial growth
T-1	Carbendazim 50%WP	1	59.2	54.2	36.24
T-2	Carbendazim 50%WP	5	38.4	33.4	60.71
T-3	Carbendazim 50%WP	10	22.0	17.0	80.00
T-4	Carbendazim 50%WP	20	5.0	0.0	100.00
T-5	Carbendazim 50%WP	25	5.0	0.0	100.00
T-6	Mancozeb 75% WP	20	70.8	65.8	22.59
T-7	Mancozeb 75% WP	50	52.8	47.8	43.76
T-8	Mancozeb 75% WP	100	21.6	16.6	80.47
T-9	Mancozeb 75% WP	200	5.0	0.0	100.00
T-10	Mancozeb 75% WP	300	5.0	0.0	100.00
T-11	Propiconazole 25% EC	1	43.0	38.0	55.29
T-12	Propiconazole 25% EC	5	25.2	20.2	76.24
T-13	Propiconazole 25% EC	10	14.0	9.0	89.41
T-14	Propiconazole 25% EC	20	5.0	0.0	100.00
T-15	Propiconazole 25% EC	25	5.0	0.0	100.00
T-16	Control Check	--	90.0	85.0	0.00
CD at 5%				1.512	
SEM ±				0.535	

Table 3: Effect of different antagonists on radial growth of test pathogen at 25 ± 2 ºC after 72 h of incubation

Sl. No.	Antagonist	Colony diameter (mm)	Actual colony diameter (mm)	Inhibition (%)
1	*T. harzianum*	52.0	47.00	44.41
2	*P. fluorescens*	55.0	50.00	41.18
3	*T. harzianum* + *P. fluorescens*	37.0	32.00	62.35
4	Check	90.0	85.00	0.00
CD at 5%				2.681
SEM ±				0.894
CV (%)				3.738

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