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In vitro evaluation of bio control agents and botanicals against mulberry root rot pathogen *Fusarium solani*

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Abstract

Mulberry is only source of food for the silkworm *Bombyx mori* L. Mulberry is susceptible to soil borne diseases among them root rot is fast spreading disease caused by *F. solani*. The pathogen can be managed by synthetic chemical fungicides but use of synthetic fungicides lead to residual toxicity effect the growth and development of silkworm. In this context, an attempt was made to use of antagonistic bio control agents for the management of this root rot pathogen. Seven fungal bio control agents and five bacterial bio control agents tested out of them *T. viridae* (Tv – B2), *T. harzianum* (Th - 44), *B. subtilis* (Bs-M) and (Bs-O) were proved as best for inhibition of mycelial growth of the pathogen. Out of eleven plant extracts used, garlic was proved best at 15 per cent and 20 per cent concentrations and agave at 20 per cent found effective against the root rot causing pathogen.

Keywords: *B. subtilis*, development, causing

1. Introduction

Mulberry (*Morus* spp.) commercially grown to feed silkworms (*Bombyx mori* L.) its only sole food for silkworm so, it is grown in different types of soils and varied climatic conditions, due to repeated harvesting of leaf soil nutrients get depleted and mulberry is susceptible to many soil borne diseases. Mulberry is affected by pathogens like fungi, bacteria, virus and nematodes. Root rot of mulberry is most important fungal disease causing considerable yield loss resulting in considerable leaf yield loss and infected plants produce nutritionally inferior leaves with reduced leaf quality (Gupta *et al.*, 1999) [2]. This root rot is most prevalent in both nurseries and established gardens (Vincent *et al.*, 1998) [11]. Among the soil-borne diseases, root rot is epidemic in nature and causes 30% mortality of plants with a 15% decrease in leaf yield, besides deteriorating the leaf quality (Rajeshwari and Angappan, 2018) [8]. In mulberry, different kinds of root rot have been reported, such as dry root rot, charcoal root rot, violet root rot, white root rot, black root rot and bacterial root rot (Gnanesh *et al.*, 2021, Radhakrishnan *et al.*, 1995, Yoshida *et al.*, 2001) [3,7,12]. Among them, dry root rot is caused by (*Fusarium solani*), charcoal root rot (*Macrophomina phaseolina*) black root rot *Lasiodiplodia theobromae* (*Botryodiplodia theobromae*) are frequently reported in India (Pinto *et al.*, 2018) [6]. This disease spreads primarily through the diseased plant samples used for propagation, contaminated soil, farm implements, irrigation water that affects all parts of the plant and it spreads rapidly affecting a large number of plants in a short period leading to the abandonment of mulberry gardens (Pappachan *et al.*, 2020) [5]. This soil borne pathogen can be managed by the use of effective fungicides but use of excess chemicals may cause residual effect on silkworm and environment pollution. Therefore, use of antagonistic fungal bio control agents like *Trichoderma* spp. and bacterial bio control agents like *Bacillus* spp., *Pseudomonas* spp. and plant extracts may help in reducing the soil borne pathogens load and these were tested *in vitro* against *Fusarium solani*.

2. Materials and Methods

The present study on *in vitro* evaluation of bio control agents and botanicals against mulberry root rot causing pathogen was carried out in the Department of plant pathology, College of Sericulture, Chintamani, University of Agricultural Sciences, Bengaluru, Karnataka, India

during 2021 - 2022. The materials used and methodology followed during the investigation are described below.

In vitro* evaluation of biocontrol agents against *F. solani

The antagonistic potential of bio agents viz., *Trichoderma harzianum*, *T. viride*., *Pseudomonas fluorescence* and *Bacillus subtilis* were tested by dual culture technique. For this 20 mL of sterilized, melted and cooled potato dextrose agar (PDA) medium was poured into each Petri plate and allowed to solidify. The plates were inoculated with 5 mm disc of 7 days old culture of fungal bio-control agents with the help of a sterilized cork borer and subsequently on opposite side inoculated with pure culture of root rot pathogens by placing a 5 mm disc of one-week old pure culture keeping 15 mm distance from the periphery. The bacterial antagonists were streaked with a sterilized inoculating loop at one end of the PDA medium Petri plates. Just opposite to the bacterial streak 5 mm disc of the pathogen was placed with a sterilized cork borer. The inoculation of pathogen alone on the center in the plates served as a control. The experiment was conducted by using Completely Randomized Design (CRD). Three replications of each treatment, including the control, were maintained. These plates were incubated at 28±1 °C. The efficacy of antagonistic organisms was recorded by measuring the colony diameter of the pathogen in each treatment and compared with control. Per cent mycelial inhibition over control was calculated by using the formula given by Vincent (1947) [11].

In vitro* evaluation of botanical extracts against *F. solani

The efficiency of plant extracts or botanical extracts was tested against root rot pathogen *F. solani* on Potato dextrose agar (PDA) medium by using poisoned food technique. For this, 100g of fresh plant parts (leaves/bulbs) were collected, washed with tap water subsequent washing with distilled water. The fresh sample was chopped and crushed by adding 100 ml sterile distilled water. The crushed product was filtered through muslin cloth. The filtrate gave 100 per cent and was used as stock solution. 5, 10 and 15 ml of stock solution was mixed with 95, 90, 85 and 80 ml of PDA medium and then it was shaken for uniform mixing of plant extract. Later, the media was sterilized and allowed to cool. Twenty ml of medium was poured into sterilized Petri plates and then fungal disc of 5 mm was placed at the center of the petri plate and incubated at 28 ± 1°C. The PDA medium without any plant extract served as control. The per cent inhibition of mycelial growth of test fungus was calculated by using following formula given by Vincent (1947) [11].

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

Where,

I = Per cent growth inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Results and discussion

In vitro* evaluation of fungal bio agents against *F. solani

The antagonistic action of selected seven fungal bio control agents against *F. solani* was carried out through dual culture technique. Based on the observation of radial growth of biocontrol agent and pathogen fungus, per cent inhibition of mycelial growth was calculated. The results are presented in Table 1, Fig. 1 and Plate 1 Among the fungal bio agents tested against *F. solani* *T. harzianum* (Th- 44) was found to be most effective and significant over other bio control agents with maximum mycelial inhibition of 69.74 per cent over control. Next in order was *T. viride* (Tv - 3) with 58.46 per cent inhibition followed by moderate inhibition observed in *T. harzianum* (Th- 55) and (Th -B2) and was on par with each other with 53.84 per cent followed by *T. viride* (Tv - B2) and *T. viride* (Tv - 2) with 51.28 and 49.74 per cent inhibition, respectively. The least mycelial inhibition was observed in *T. viride* (Tv - 5) with 47.17 per cent inhibition, respectively.

The inhibitory effect of these fungal biocontrol agents may be due to hyper parasitism, competition for space and nutrients or antibiotics. The findings are in confirmation with the studies conducted by Narayanan *et al.* (2015) [4] who reported the efficacy of potential biocontrol agents and fungicides against mulberry wilt caused by *F. solani*. Three antagonists viz., *Trichoderma viride*., *Pseudomonas fluorescens* were evaluated and the results showed that *Trichoderma* significantly reduced the mycelial growth of the pathogen. In pot culture studies, the minimum (10.5 %) incidence of wilt was observed in soil drenching (Seri bed waste+Pfl+Bs4+Tv1+Neem cake) which showed 12.3 per cent incidence as compared to maximum (46.7 %) wilt incidence in control.

Table 1: *In vitro* evaluation of fungal bio agents against *F. solani*

| Sl. No. | Fungal Bio-agents | Isolate | Per cent inhibition of mycelial growth (%) |
|---------|------------------------------|---------|--|
| | | | <i>F. solani</i> |
| 1 | <i>Trichoderma harzianum</i> | Th- B2 | 53.84 (47.18) * |
| 2 | <i>T. harzianum</i> | Th-55 | 53.84 (47.18) |
| 3 | <i>T. harzianum</i> | Th-44 | 69.74 (56.66) |
| 4 | <i>T. viride</i> | Tv-2 | 49.74 (44.86) |
| 5 | <i>T. viride</i> | Tv-B2 | 51.28 (45.71) |
| 6 | <i>T. viride</i> | Tv-3 | 58.46 (49.86) |
| 7 | <i>T. viride</i> | Tv-5 | 47.17 (43.36) |
| 8 | Control | - | 0.00 (0.00) |
| | F test | | * |
| | S. Em± | | 2.478 |
| | CD @1% | | 6.64 |

* Figures in the parentheses are arcsine transformed values

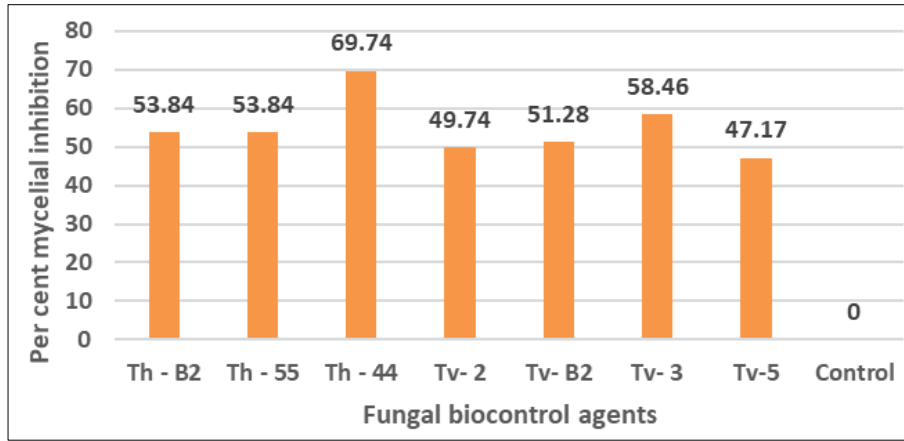


Fig 1: Effect of fungal bio agents against *F. solani*



Plate 1: *In vitro* evaluation of fungal bio control agents against *F. solani*.

3.2 *In vitro* evaluation of bacterial bio agents against *F. solani*: The antagonistic action of selected bacterial bio control agents against *F. solani* was tested through dual culture technique. Based on the observations of radial growth of the bio agents and fungus, the per cent inhibition was calculated. The results are presented in Table 2, Fig.2 and Plate 2. Among the bacterial bio agents tested against *F. solani* the *Bacillus subtilis* (Bs-M) was significantly superior over control with 39.62 per cent mycelial inhibition. This is followed by *B. subtilis* (Bs-O) with 34.44 per cent mycelial inhibition followed by *P. fluorescense* (Pf-O) with 25.92 per cent mycelial inhibition, followed by *B. subtilis* (Bs-P) with 6.21 per cent. The least inhibition among all was found in *P. fluoscense* (Pf - C) with 4.44 per cent.

These findings were similar to the results of Sundaramoorthy *et al.* (2012) [10] who evaluated the protective effects of compatible endophytic bacterial strains (*Bacillus subtilis*; EPCO16 and EPC5) and *Rhizobacterial* strain (*Pseudomonas fluorescens*; Pf1) against wilt disease caused by *Fusarium solani*. The results showed that *B. subtilis* (EPCO16 and EPC5) and *P. fluorescens* (Pf1) were compatible and effectively inhibited the growth of the *F.*

solani. Similarly, Seetha *et al.* (2010) [9] screened different bio control agents under *in vitro* conditions and found that *Pseudomonas fluorescens* and *Trichoderma viride* showed maximum inhibition of the mycelial growth (95%) against *F. solani* followed by *P. fluorescens* (90%). and *T. harzianum* (80%).

Table 2: *In vitro* evaluation of bacterial bio agents against *F. solani*

| Sl. No. | Bacterial bio agent | Isolate | Per cent inhibition of mycelial (%) |
|---------|---------------------------------|---------|-------------------------------------|
| | | | <i>F. solani</i> |
| 1 | <i>Bacillus subtilis</i> | Bs - P | 6.29 (13.88) |
| 2 | <i>Bacillus subtilis</i> | Bs -M | 39.62 (38.99) |
| 3 | <i>Bacillus subtilis</i> | Bs- O | 34.44 (39.33) |
| 4 | <i>Pseudomonas fluorescense</i> | Pf -O | 25.92 (30.37) |
| 5 | <i>Pseudomonas fluorescense</i> | Pf - C | 4.44 (11.95) |
| 6 | Control | - | 0.00 (0.00) |
| | F test | | * |
| | S. Em± | | 4.185 |
| | CD @ 1% | | 13.358 |

* Figures in the parentheses are arcsine transformed values

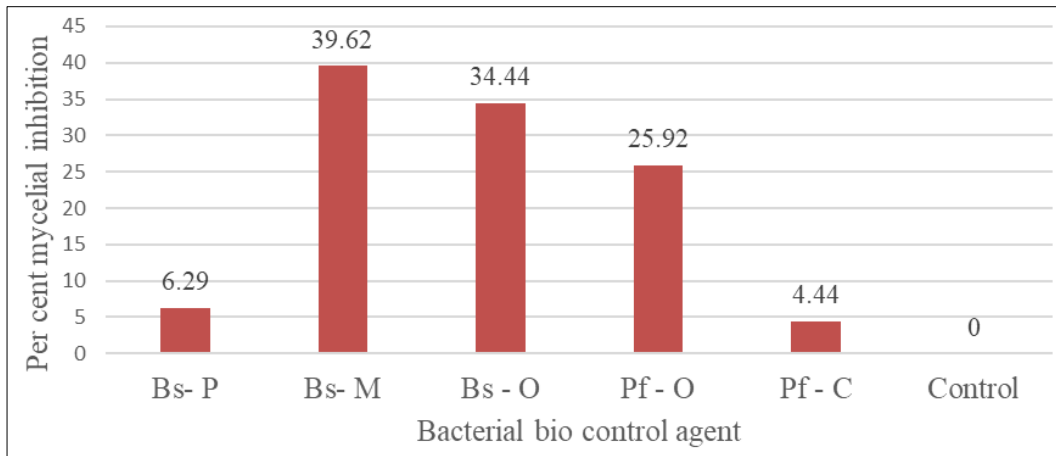


Fig 2: Effect of bacterial bio agents against *F. solani*.

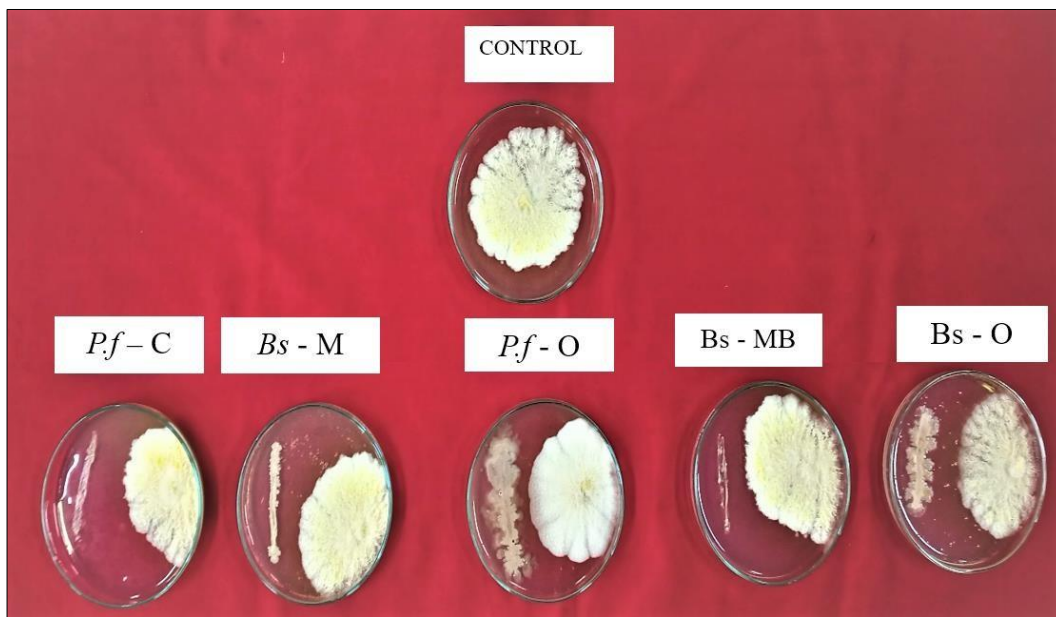


Plate 2: *In vitro* evaluation of bacterial bio agents against *F. solani*.

3.3 *In vitro* evaluation of botanical extracts against *F. solani*

Eleven botanical extracts were tested at four concentrations viz., 5, 10, 15 and 20 per cent by using poisoned food technique under *in vitro* condition. The per cent inhibition of mycelial growth of *F. solani* in different botanicals are presented in (Table 3, Fig.3 and Plate 3). Out of eleven botanicals tested against *F. solani*, garlic extract significantly inhibited the mycelial growth (68.62 %). Out of four concentrations 15 and 20 per cent inhibited cent per cent and 26.47 and 48.04 per cent inhibition in 5 and 10 per cent, respectively. Among the four concentrations of agave extract mycelial inhibition was 19.61, 27.45, 28.92 and 31.86 per cent was observed in 5, 10, 15 and 20 per cent concentrations respectively. Neem recorded the mean mycelial inhibition of 21.49 per cent. Further, 25.57 per cent mycelial inhibition observed in 20 percent 17.65, 20.10 and 21.57 per cent mycelial inhibition 5, 10 and 15 per cent concentration in pongemia extract. The remaining botanicals showed moderate inhibition ranging from 15.68 to 20.47 per cent of mycelial inhibition. The onion extract inhibited 20.47 per cent of mean mycelial inhibition. Among four concentrations of 5, 10, 15 and 20 per cent mycelial

inhibition of 16.67, 17.65, 20.10 and 25.00 per cent was observed in onion extract. The mycelial inhibition in agave, Simaruba, Tulsi and pongemia extracts were 19.49, 19.36, 19.12, 19.00 per cent, respectively. The ginger extract recorded 15.44 per cent inhibition. Least per cent inhibition was observed in touch me not with 15.69 per cent.

Similar findings were observed in studies conducted by Seetha *et al.* (2010)^[9] where ten plant extracts were tested against fungal pathogens under *in vitro* through poisoned food technique. Among different Plant extract evaluated *Prosopis juliflora* showed the maximum inhibition of mycelial growth of 80.0 per cent over *F. solani* followed by *Lantana camara* (68.90%). Similarly, Babu *et al.* (2008)^[11] evaluated different botanicals against *F. solani* f. sp. melongenae. The *in vitro* efficacy of different plant extracts viz., *Azardiachta indica*, *Artemessia annua*, *Eucalyptus globulus*; *Ocimum sanctum* and *Rheum emodi* in different concentration were tested to control brinjal wilt pathogen. All the plant extracts showed significant reduction in the growth of pathogen. Among the different extracts, *Azardiachta indica* (20%) was found most effective followed by *Rheum emodi*, *Eucalyptus globulus*, *Artemessia annua* and *Ocimum sanctum*.

Table 7: *In vitro* evaluation of botanical extracts against *F. solani*

| Sl. No. | Botanicals Common name | Per cent mycelial inhibition (%) | | | | Mean mycelial inhibition (%) |
|---------|---------------------------|----------------------------------|---------------|-------------------|----------------|------------------------------|
| | | Concentrations | | | | |
| | | 5% | 10% | 15% | 20% | |
| 1 | Neem | 17.16 (24.37) | 20.01 (26.62) | 21.57 (27.64) | 25.49 (30.30) | 21.08 (27.23) |
| 2 | Subabul | 19.61 (26.25) | 27.45 (31.56) | 28.92 (32.50) | 31.86 (34.34) | 26.96(31.16) |
| 3 | Lemon grass | 14.22 (22.06) | 15.67 (23.31) | 17.65 (24.81) | 23.04 (28.59) | 17.65 (24.69) |
| 4 | Ginger | 9.80 (18.04) | 14.71 (22.54) | 16.18 (23.69) | 21.08 (27.30) | 15.44 (22.86) |
| 5 | Garlic | 26.47 (30.90) | 48.04 (43.85) | 100.00 (90.00) | 100.00 (90.00) | 68.63 (63.67) |
| 6 | Pongemia | 16.67 (23.96) | 17.65 (24.66) | 20.01 (26.62) | 21.57 (27.659) | 18.99 (25.72) |
| 7 | Simaruba | 15.19 (22.70) | 19.61 (26.14) | 21.08 (27.26) | 21.57 (27.62) | 19.36 (25.93) |
| 8 | Onion | 16.67 (24.08) | 19.12 (25.90) | 21.08 (27.27) | 25.00 (29.92) | 20.47 (26.81) |
| 9 | Tulsi | 13.23 (21.30) | 17.65 (24.81) | 20.01 (26.61) | 25.49 (30.30) | 19.19 (25.76) |
| 10 | Agave | 15.11 (22.89) | 17.16 (24.81) | 20.01 (26.60) | 25.49 (30.30) | 19.48 (26.06) |
| 11 | Touch me not | 5.88 (13.95) | 10.78 (19.03) | 21.08 (27.23) | 25.00 (29.98) | 15.69 (22.55) |
| 12 | Control | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | Mean | 14.17 (20.87) | 18.98 (24.40) | 25.64 (30.01) | 28.80 (32.29) | 21.90 (26.87) |
| | | Botanical extracts (B) | | Concentration (C) | | Interaction (B × C) |
| | F test | * | | * | | * |
| | S. Em± | 0.78 | | 0.4 | | 1.564 |
| | CD at 1% | 2.20 | | 1.30 | | 4.44 |

* Figures in the parenthesis are arcsine transformed values

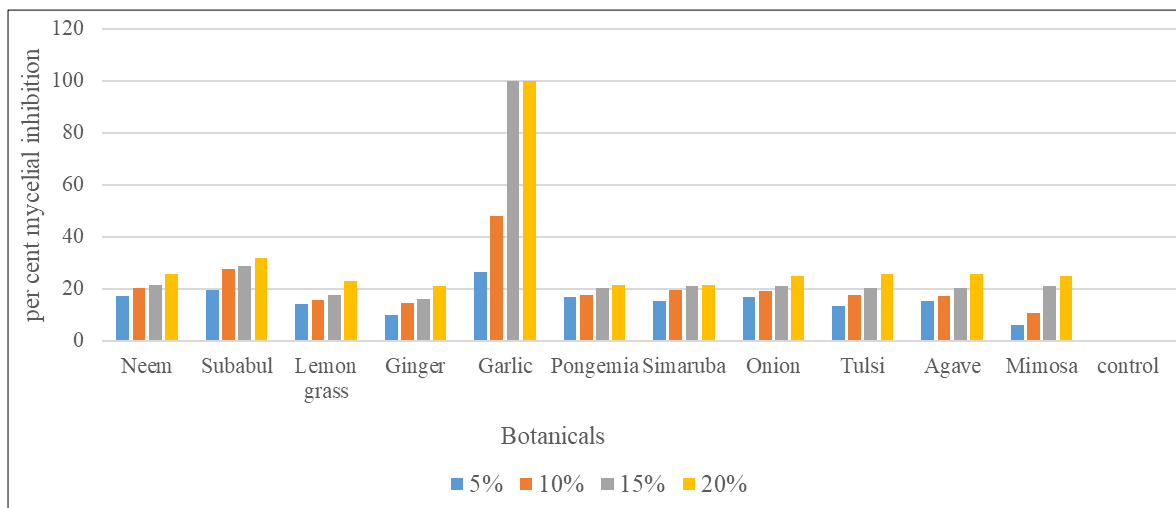
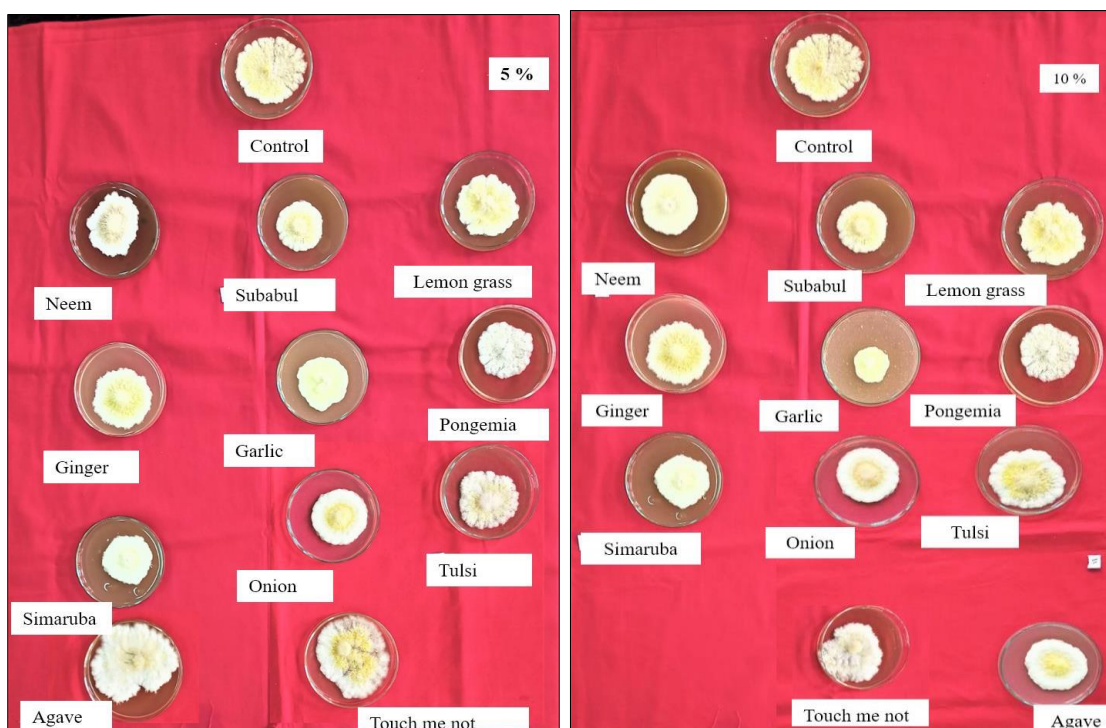


Fig 4: Effect of Botanical extracts against *F. solani*



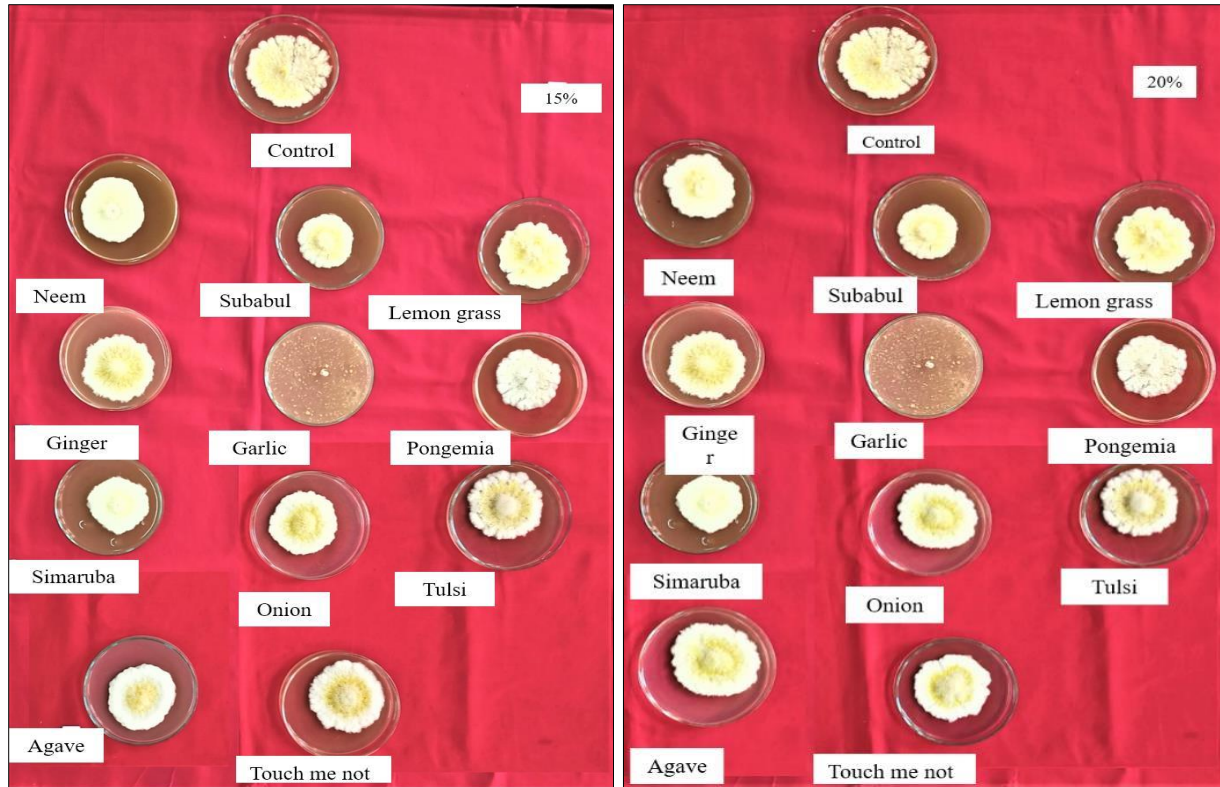


Plate 3: *In vitro* evaluation of botanical extracts against *F. solani*.

4. Conclusion

seven fungal bio agents and five bacterial bio control agents were evaluated *In vitro* against *F. solani*. out of them *T. harzianum* (Th - 44), *T. viridae* (Tv - 3), *B. subtilis* (Bs - M) and (Bs - O) were proved as best for inhibition of mycelial growth of the pathogens. Out of eleven plant extracts tested garlic was best with cent percent mycelial inhibition at 15 and 20 per cent concentrations and proved best for the inhibition of the root rot disease.

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