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# Bioefficacy of Bioagents and Phytoextracts against *Phyllosticta zingiberi*, causing *Phyllosticta* leaf spot of ginger in *In vitro* conditions

**Neha NP, AP Suryawanshi, PD Biradar, VR Wadhave and MR Kharade**

**Abstract**
Ginger (*Zingiber officinale*) crop is affected by several pathogens/diseases, of which *Phyllosticta* leaf spot (*Phyllosticta zingiberi*) is dreaded fungal disease, causing accountable quantitative and qualitative losses. Therefore, present study was attempted to assess the bioefficacy of nine each bioagents and acetone phytoextracts against *P. zingiberi*.

The results revealed all of the test biocontrol agents as potential antagonists, which significantly inhibited mycelial growth of *P. zingiberi*, over untreated control. However *Trichoderma hamatum* resulted with significantly highest mycelial inhibition (87.77%), followed by *T. koningii* and *Metarhizium anisopliae* (76.66%), *Verticillium lecanii* (71.48%), *T. asperellum* (65.55%), *T. harzianum* (55.18%), *Aspergillus niger* (46.66%) and *Pseudomonas fluorescens* (44.44%)

All nine test phytoextracts (each @10 and 20%) were found fungistatic to *P. zingiberi* and significantly inhibited mycelial growth of the test pathogen, over untreated control. Among phytoextracts *Lawsonia inermis* resulted with significantly highest mycelial growth inhibition (52.59 and 66.66%), followed by *Allium sativum* L. (32.59 and 36.66%), *Azadirachta indica* (24.81 and 28.14%), *Zingiber officinale* (23.70 and 32.22%) and *Allium cepa* (22.59 and 26.66%), respectively @ 10 and 20%.

**Keywords:** *Zingiber officinale*, *Phyllosticta zingiberi*, Bioagents, Phytoextracts

## Introduction
Ginger (*Zingiber officinale*), an herbaceous perennial plant belonging to the family Zingiberaceae, is commonly known as Sringavera in Sanskrit, Zingiberi in Greek and Zingiber in Latin (Vasala, 2004) [13]. It is probably the native of South-East Asia and can also be grown in other parts of the world (Govindrajan, 1982 a, b). It is one of the most important spice crop traded internationally and domestically for the purpose of spices, medicines, foods like salted ginger and beverages. India is the world's leading producer of ginger (Medhi *et al*., 2012) [7].

Ginger crop suffers from many insect pests and biotic / abiotic stresses. Among biotic stresses, the diseases caused by fungi, bacteria, viruses and nematodes, are the major constraints in ginger production and productivity. Among major diseases of ginger, *Phyllosticta* leaf spot (*P. zingiberi*) had been reported as most destructive and causes severe damage of foliage which ultimately results in significant yield losses (Sohi *et al.*, 1964; Sood and Dohroo, 2005; Singh, 2015) [10, 11, 9].

The *Phyllosticta* spp. are one of the widely distributed phytopathogens infecting a wide range of agronomical, ornamental and horticultural crop plant species, cultivated worldwide. In *Phyllosticta* spp., the existence of a high level of variability *viz*., cultural, morphological and pathogenic have earlier been reported by several workers (Sood, 2002; Sood and Dohroo, 2005; Wikee *et al.*, 2013, Yi *et al*., 2015, Malali, 2018) [12, 11, 14, 15, 6]. *Phyllosticta* leaf spot (*Phyllosticta zingiberi*) is dreaded fungal disease, causing accountable quantitative and qualitative losses. Therefore, present study was attempted to assess the bioefficacy of nine each bioagents and acetone phytoextracts against *P. zingiberi*

## Materials and Methods
### 1. *In vitro* evaluation of bioagents
A total of eight most potential bioagents (fungal and bacterial) were evaluated *in vitro* against *P. zingiberi* test isolate (Pz-Kh), by applying 'Dual Culture Technique' (Dennis and Webster, 1971) and using PDA as basal culture medium. Seven day aged cultures of the test bioagents and test pathogen, grown on respective culture media were used for the study. One each

culture disc (5 mm) of the test pathogen and the test fungal bioagent (cut out with sterilized cork borer) were placed at equidistance and exactly opposite to each other, on autoclaved and solidified PDA medium in Petri plates. For bacterial bioagents, a culture disc (5 mm) of the test pathogen was placed along periphery of the PDA plate and exactly opposite to it pure culture suspension of the test bacterial bioagent streaked with wire / inoculation needle loop. For each test bioagent, three PDA plates were inoculated and all the treatments replicated thrice. The PDA plates inoculated (in the centre) only with pure culture disc of the test pathogen were maintained as untreated control.

**List of the bioagents**

| Tr. No. | Treatments | Tr. No. | Treatments |
|---|---|---|---|
| $T_1$ | *Trichoderma asperellum* | $T_6$ | *Metarhizium anisopliae* |
| $T_2$ | *T. harzianum* | $T_7$ | *Verticillium lecanii* |
| $T_3$ | *T. hamatum* | $T_8$ | *Bacillus subtilis* |
| $T_4$ | *T. koningii* | $T_9$ | *Pseudomonas fluorescens* |
| $T_5$ | *Aspergillus niger* | $T_{10}$ | Control (untreated) |

Observations on linear colony growth / diameter (mm) of the test pathogen and the test bioagent were recorded at an interval of 24 hrs of incubation and continued upto seven days or till the untreated control plates were fully covered with mycelial growth of the test pathogen. Based on cumulative data, per cent mycelial growth inhibition of the test pathogen with the test bioagents over untreated control was calculated by applying following formula (Arora and Upadhay, 1978) [2].

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

**2. *In vitro* evaluation of phytoextracts**

Solvent (acetone) extracts of the nine locally available higher plant species were separately evaluated *in vitro* (each @ 10 and 20 %) against *P. zingiberi* test isolate (Pz-Kh), using PDA as a basal culture medium and by applying Poisoned food technique (Nene and Thapliyal, 1993) [8].

Plant parts (leaves, bulbs, cloves, rhizomes etc.) of the selected plant species were washed thoroughly under running tap water, air dried and chopped into small bits with sterilized sharp knife. Then these were separately crushed and homogenized using pestle and mortar, in an equal quantity of distilled water (1:1 w/v). The homogenates obtained were initially strained through double layered muslin cloth and the extracts / filtrates obtained were further filtered through Whatman No. 1 filter paper using glass volumetric flasks (100 ml capacity) and funnel, which yielded clear phytoextracts of 100 per cent concentration.

An appropriate quantity of each test solvent phytoextracts (100 %) was separately mixed thoroughly with autoclaved and cooled (45° C) PDA medium in conical flasks (250 ml), to prepare separately the phytoextract amended PDA medium of 10 and 20 per cent concentration. This PDA medium amended separately with the test solvent extract was then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. Three PDA plates per test phytoextract per replication were maintained and replicated thrice. Upon solidification of the amended PDA medium, all these treatment plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc, obtained from a week aged actively growing pure culture of the test pathogen isolate (Pz-Kh). Plain PDA plates without any plant extract and inoculated with mycelial disc of the test pathogen were maintained as untreated control. Then, these PDA plates were incubated at 27 ± 2 $^0$C temperature for a week or until the untreated control plates were fully covered with mycelial growth of the test pathogen.

**List of the phytoextracts**

| Tr. No. | Treatments | Plant parts used | Tr. No. | Treatments | Plant parts used |
|---|---|---|---|---|---|
| $T_1$ | *Eucalyptus globulus* (Nilgiri) | Leaves | $T_6$ | *Pongamia pinnata* (Karanj) | Leaves |
| $T_2$ | *Allium cepa* (Onion) | Bulb | $T_7$ | *Zingiber officinale* (Ginger) | Rhizome |
| $T_3$ | *Allium sativum* L. (Garlic) | Clove | $T_8$ | *Aloe vera* (Korphad) | Gel |
| $T_4$ | *Azadirachta indica* (Neem) | Leaves | $T_9$ | *Lawsonia inermis* (Mehandi) | Leaves |
| $T_5$ | *Lantana camara* L. (Ghaneri) | Leaves | $T_{10}$ | Control (untreated) | |

Observations on radial mycelial growth / colony diameter (mm) were recorded at an interval of 24 hrs. of incubation and continued upto seven days of incubation or till the untreated PDA plates were fully covered with mycelial growth of the test pathogen. Based on cumulative data, per cent mycelial growth inhibition of the test pathogen with the test phytoextracts, over untreated control was calculated by applying following formula (Vincent, 1927) [16].

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates

**Results and Discussion**

**1. *In vitro* efficacy of bioagents against *Phyllosticta zingiberi*, causing leaf spot of ginger**

Results (Table 1, Fig 1, Plate 1) revealed that all the bioagents evaluated *in vitro* exhibited antifungal activity against *P. zingiberi* (isolate Pz-Kh) and numerically inhibited its growth, over untreated control.

**Table 1:** *In vitro* efficacy of bioagents against *P. zingiberi* (isolate Pz-Kh), causing leaf spot of ginger

| Tr. No. | Treatments | Colony Diam.* of test pathogen (mm) | Av. Inhibition* (%) |
|---|---|---|---|
| $T_1$ | *Trichoderma asperellum* | 31.00 | 65.55 (54.04) |
| $T_2$ | *T. harzianum* | 40.33 | 55.18 (47.95) |
| $T_3$ | *T. hamatum* | 11.00 | 87.77 (69.51) |
| $T_4$ | *T. koningii* | 21.00 | 76.66 (61.09) |
| $T_5$ | *Aspergillus niger* | 48.00 | 46.66 (43.06) |
| $T_6$ | *Metarhizium anisopliae* | 21.00 | 76.66 (61.09) |
| $T_7$ | *Verticillium lecanii* | 25.66 | 71.48 (57.69) |
| $T_8$ | *Bacillus subtilis* | 81.00 | 9.99 (18.40) |
| $T_9$ | *Pseudomonas fluorescens* | 50.00 | 44.44 (41.79) |
| $T_{10}$ | Control (untreated) | 90.00 | 00.00 (00.00) |
| | S.E.± | 0.56 | 0.63 |
| | C.D. (P=0.01) | 1.68 | 1.87 |

*Mean of three replications. Figures in parentheses are parentheses are arcsine transformed values. Diam.: Diameter, Av.: Average

The results (Table 1, Fig 1, Plate 1) revealed *T. hamatum* as most effective bioagent with significantly least mycelial growth (11.00 mm) and significantly highest mycelial inhibition (87.77 %), followed by *T. koningii* and *Metarhizium anisopliae* (21.00 mm and 76.66 %), *Verticillium lecanii* (25.66 mm and 71.48 %), *Trichoderma asperellum* (31.00 mm and 65.55 %), *T. harzianum* (40.33 mm and 55.18 %) *Aspergillus niger* (48.88 mm and 46.66 %), *Pseudomonas fluorescens* (50.00 mm and 44.44 %) and *Bacillus subtilis* (81.00 mm and 9.99 %).

These test bioagents were reported as efficient antagonists against many *Phyllosticta* spp. including *P. zingiberi* by several earlier workers (Sood, 2002; Arunakumara and Satyanarayana, 2015; Mathalemuse and Kena, 2017) [12, 1].

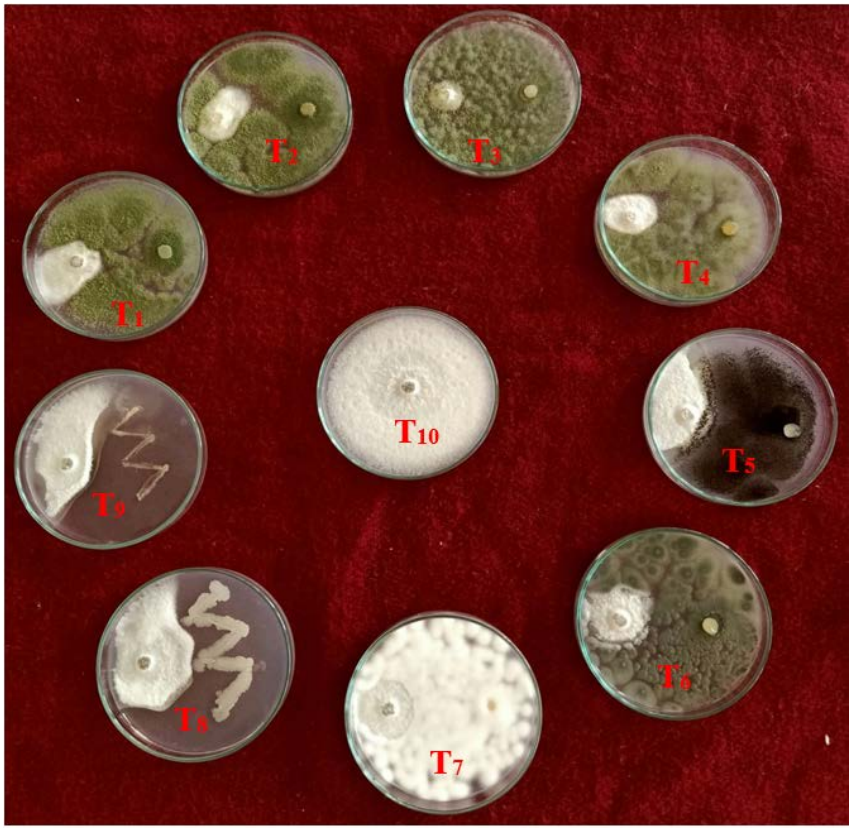


**Plate 1:** *In vitro* efficacy of various bioagents against *P. zingiberi*, causing leaf spot of ginger

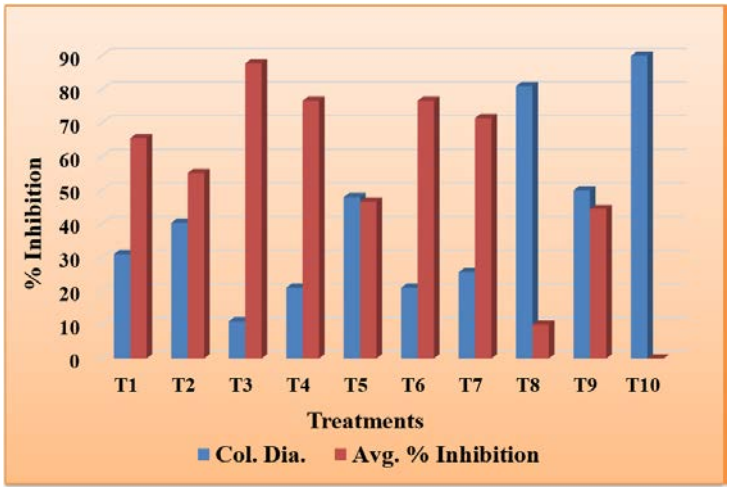


**Fig 1:** *In vitro* efficacy of bioagents against *P. zingiberi*, causing leaf spot of ginger

**2. *In vitro* efficacy of phytoextracts against *Phyllosticta zingiberi*, causing leaf spot of ginger**

Solvent extracts (leaf / bulb / rhizome) of nine plant species were evaluated *in vitro* (each @ 10 and 20 %) against *P. zingiberi* (isolate Pz-Kh) and the results obtained on mycelial growth and its inhibition are presented (Table 2 and Fig. 2 Plate 2)

**Table 2:** *In vitro* efficacy of phyto extracts against *P. zingiberi* (isolate Pz-Kh), causing leaf spot of ginger

| Tr. No. | Treatments | Colony diam.* (mm) | | Av. (mm) | % Inhibition* | | Av. Inhibition (%) |
|---|---|---|---|---|---|---|---|
| | | 10 % | 20 % | | 10 % | 20 % | |
| $T_1$ | *Eucalyptus globulus* | 76.66 | 73.00 | 74.83 | 14.81 (22.60) | 18.88 (25.74) | 16.84 (23.90) |
| $T_2$ | *Allium cepa* | 69.66 | 66.00 | 67.83 | 22.59 (28.35) | 26.66 (31.07) | 24.62 (29.71) |
| $T_3$ | *Allium sativum* | 60.66 | 57.00 | 58.83 | 32.59 (34.79) | 36.66 (37.24) | 34.62 (36.01) |
| $T_4$ | *Azadirachta indica* | 67.66 | 64.66 | 66.16 | 24.81 (29.85) | 28.14 (32.02) | 26.47 (30.93) |
| $T_5$ | *Lantana camara* | 74.66 | 69.66 | 72.16 | 17.03 (24.34) | 22.59 (28.36) | 21.31 (26.35) |
| $T_6$ | *Pongamia pinnata* | 72.00 | 67.00 | 69.50 | 19.99 (26.54) | 25.55 (30.34) | 22.77 (28.44) |
| $T_7$ | *Zingiber officinale* | 68.66 | 61.00 | 64.83 | 23.70 (29.11) | 32.22 (34.56) | 27.96 (31.83) |
| $T_8$ | *Aloe barbadensis* | 72.33 | 66.00 | 69.16 | 19.62 (26.28) | 26.66 (31.07) | 23.14 (28.67) |
| $T_9$ | *Lawsonia inermis* | 42.66 | 30.00 | 36.33 | 52.59 (46.46) | 66.66 (54.71) | 59.62 (50.58) |
| $T_{10}$ | Control (untreated) | 90.00 | 90.00 | 90.00 | 00.00 (00.00) | 00.00 (00.00) | 00.00 (00.00) |
| | S.E. ± | 0.76 | 0.50 | - | 0.85 | 0.56 | - |
| | C.D. (P=0.01) | 2.28 | 1.50 | - | 2.53 | 1.66 | - |

*Mean of three replications. Figures in parentheses are parentheses are arcsine transformed values. Diam.: Diameter, Av.: Average


**Plate 2:** *In vitro* efficacy of various bioagents against *P. zingiberi*, causing leaf spot of ginger

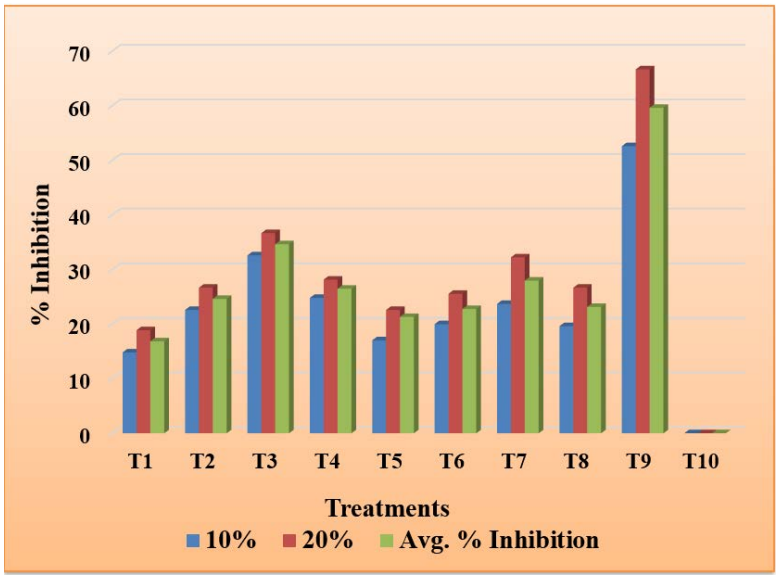


**Fig 2:** *In vitro* efficacy of phytoextracts against *P. zingiberi*, causing leaf spot of ginger

**Effect on mycelial growth**

At 10 per cent, radial mycelial growth of *P. zingiberi* (isolate Pz-Kh) ranged from 42.66 mm to 76.66 mm. However, it was numerically least with *Lawsonia inermis* (42.66 mm), followed by *Allium sativum* L. (60.66 mm), *Azadirachta indica* (67.66 mm), *Zingiber officinale* (68.66 mm), *Allium cepa* (69.66 mm), *Pongamia pinnata* (72.00 mm), *Aloe barbadensis* (72.33 mm), *Lantana camara* L. (74.66 mm) and *Eucalyptus globulus* (76.66 mm).

At 20 per cent, radial mycelial growth of the test pathogen was ranged from 30.00mm to 73.00 mm. However, it was numerically least with *Lawsonia inermis* (30.00 mm), followed by *Allium sativum* L.(57.00 mm), *Zingiber officinale* (61.00 mm), *Azadirachta indica* (64.66 mm), *Allium cepa* and *Aloe barbadensis* (66.00 mm), *Pongamia pinnata* (67.00 mm), *Lantana camara* L. (69.66 mm) and *Eucalyptus globulus* (73.00 mm).

Average mycelial growth ranged from 36.33 mm to 74.83 mm. However, it was numerically least with *Lawsonia inermis* (36.33 mm), followed by *Allium sativum* L. (58.83 mm), *Zingiber officinale* (64.83 mm), *Azadirachta indica* (66.16 mm), *Allium cepa* (67.83 mm), *Aloe barbadensis* (69.16 mm), *Pongamia pinnata* (69.50 mm), *Lantana camara* (72.16 mm) and *Eucalyptus globulus* (74.83 mm).

**Effect on mycelial growth inhibition**

Results (Table 2 and Fig. 2 Plate 2) revealed that the phytoextracts tested numerically inhibited mycelial growth of *P. zingiberi*, over untreated control and it was increased with increase in concentration of the phytoextracts tested.

At 10 per cent, mycelial growth inhibition of *P. zingiberi* (isolate Pz-Kh) ranged from 14.81 % to 52.59 %. However, it was numerically highest with *Lawsonia inermis* (52.59 %), followed by *Allium sativum* L. (32.59 %), *Azadirachta indica* (24.81 %), *Zingiber officinale* (23.70 %), *Allium cepa* (22.59 %), *Pongamia pinnata* (19.99 %), *Aloe barbadensis* (19.62 %), *Lantana camara* L. (17.03 %) and *Eucalyptus globulus* (14.81 %).

At 20 per cent, mycelial growth inhibition of *P. zingiberi* ranged from 18.88 % to 66.66 %. However, it was numerically highest with *Lawsonia inermis* (66.66 %), followed by *Allium sativum* L. (36.66 %), *Zingiber officinale* (32.22 %), *Azadirachta indica* (28.14 %), *Allium cepa* and *Aloe barbadensis* (26.66 %), *Pongamia pinnata* (25.55 %), *Lantana camara* L. (22.59 %) and *Eucalyptus globulus* (18.88 %).

Average mycelial growth inhibition of *P. zingiberi* ranged from 16.84 % to 59.62 %. However, it was numerically highest with *Lawsonia inermis* (59.62 %), followed by *Allium sativum* L. (34.62 %), *Zingiber officinale* (27.96 %), *Azadirachta indica* (26.47 %), *Allium cepa* (24.62 %), *Aloe barbadensis* (23.14 %), *Pongamia pinnata* (22.77 %), *Lantana camara* (21.31 %) and *Eucalyptus globulus* (16.84 %).

These results of the present study on antifungal potential of the test phytoextracts are in consonance with the finding of several earlier workers. The phytoextracts *viz.*, *Allium sativum* L., *Zingiber officinale*, *Azadirachta indica*, *Allium cepa*, *Aloe barbadensis*, *Pongamia pinnata*, *Lantana camara* and *Eucalyptus globulus* were reported antifungal / fungistatic against many *P. zingiberi* earlier by many workers (Sood, 2002; Arunakumara and Satyanarayana, 2015) [12, 1].

**Conclusion**

It is concluded from above result that, among the bioagents tested *in vitro*, *Trichoderma hamatum*, *T. koningii*, *Metarhizium anisopliae*, *Verticillium lecanii* and *T. asperellum* were proved to be most potent antagonists of *P. zingiberi.* Among the phytoextracts evaluated *in vitro*, *Lawsonia inermis*, *Allium sativum* L., *Zingiber officinale*, *Azadirachta indica and Allium cepa* were proved to be most efficient in inhibiting significant mycelial growth of *P. zingiberi.* Thus, locally available plant species with antimicrobial properties and antagonistic microorganisms can be used as an alternative to the chemicals, to manage *Phyllosticta* leaf spot of ginger.

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