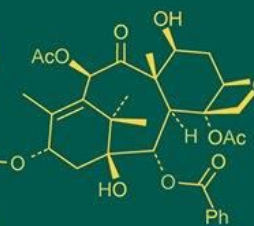
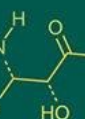
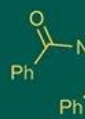
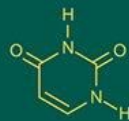
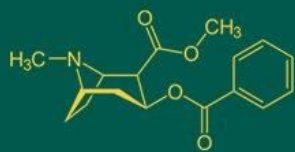


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Arati V Gajera
Department of Plant
Pathology, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Keshavji K Kanzaria
Department of Plant
Pathology, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Shruti G Solanki
Department of Plant
Pathology, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Divya G Pithiya
Department of Plant
Pathology, Junagadh
Agricultural University,
Junagadh, Gujarat, India

In vitro evaluation of different phytoextracts and biocontrol agents against *Alternaria alternata* (Fr.) Keissler causing Alternaria leaf blight of isabgol

Arati V Gajera, Keshavji K Kanzaria, Shruti G Solanki and Divya G Pithiya

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Abstract

Isabgol (*Plantago ovata* Forsk.) is known to affect by many fungal diseases. Among them, *Alternaria* leaf blight caused by *Alternaria alternata* (Fr.) Keissler has become a serious problem of isabgol in recent years in Saurashtra region of Gujarat state. Looking to the importance of disease, attempts were made to evaluate the efficacy of different ten phytoextracts, six fungal and six bacterial biocontrol agents against *A. alternata in vitro*. The results revealed that among ten phytoextracts maximum mycelial growth inhibition was observed in rhizome extract of *Zingiber officinale* Rosc. (65.42%), but it was remained statistically at par with leaf extract of *Calotropis gigantea* L. (64.68%), bulb extract of *Allium cepa* L. (63.94%) and clove extract of *Allium sativum* L. (62.83%) each at 10 per cent concentration. While, minimum mycelial growth inhibition of 9.02 per cent was observed in bulb extract of *Allium cepa* L. followed by leaf extract of *Aegle marmelos* L. (12.57%) each at 5 per cent concentration. *Trichoderma harzianum* JAU isolate (2×10^6 cfu/g min.) among fungal biocontrol agent gave maximum mycelial growth inhibition of 47.02 per cent *in vitro*. While, *Trichoderma viride* JAU isolate (2×10^6 cfu/g min.) remained least effective treatment with 14.06 per cent mycelial growth inhibition. *Bacillus amyloliquefaciens* NAU isolate (1×10^8 cfu/ml min.) on the other hand exhibited maximum mycelial growth inhibition of 44.82 per cent among bacterial biocontrol agents. But, it was remained statistically at par with *Pseudomonas fluorescens* JAU isolate 1 (1×10^8 cfu/ml min.) with 44.10 per cent mycelial growth inhibition. Whereas, *Bacillus subtilis* JAU isolate 1 (1×10^8 cfu/ml min.) showed least effective treatment with 27.40 per cent mycelial growth inhibition of *A. alternata* under laboratory condition.

Keywords: Isabgol, *Alternaria alternata*, Alternaria leaf blight, phytoextracts, biocontrol agents, mycelial growth inhibition

1. Introduction

Isabgol (*Plantago ovata* Forsk.) is a valuable medicinal crop that belongs to the family Plantaginaceae with a chromosome number of $2n=8$. It is known by various names as "Blond Psyllium in English" and "Shlakshnajira Shignabya" in Sanskrit. *P. ovata* commonly known as isabgol and locally as "Ghodajiru" or "Uthmujiru" in Gujarat. The genus *Plantago* includes 200 species (Rahn, 1996) [14], out of which ten occurs in India. Among them, three important species are viz., *ovata*, *psyllium* and *indica*. However, *P. ovata* is the only cultivated species in the country because of its bold seeds and easy dehusking property. The crop is mainly cultivated in the states of Gujarat, Rajasthan, Haryana and Madhya Pradesh. India ranks first in isabgol production (98%) and is the sole supplier of seeds and husk in the international market. Gujarat commands near monopoly in the production and export of isabgol seeds and seed husk to the world market. Isabgol husk is the only part of the plant used in manufacturing psyllium products. Psyllium fiber is widely used as a laxative agent, to treat hypercholesterolemia and to reduce the blood glucose. In pharmaceutical industry, psyllium is used as thickening agent.

The crop is known to infect by number of fungal diseases like, wilt (*Fusarium oxysporum* Schlecht. Snyd. Et Hans.), damping off (*Pythium ultimum* Trow.), leaf blight (*Alternaria alternata* (Fr.) Keissler), downy mildew (*Peronospora plantaginis* Underwood, *Peronospora alta* Fuckel and *Pseudoperonospora plantaginis*) and powdery mildew caused by *Erysiphe cichoracearum* D. C. (Mandal, 2010) [9]. Among them, Alternaria leaf blight caused by

Corresponding Author:
Arati V Gajera
Department of Plant
Pathology, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Alternaria alternata (Fr.) Keissler has become a serious problem for isabgol cultivation in recent years in Saurashtra affecting seed quality and quantity. It causes considerable damage to the crop and sometimes become severe which results in near total loss of yield under field condition. Considering the importance of the isabgol crop and regular occurrence of the disease in moderate to severe form in Saurashtra region of Gujarat state, seriousness of the disease and economic importance of the crop, it is very necessary to develop an effective ecofriendly management strategy. Hence, the present study was framed in order to contribute new insights into disease management strategy against *Alternaria alternata* (Fr.) Keissler causing Alternaria leaf blight of isabgol using phytoextracts, fungal and bacterial biocontrol agents under *in vitro* condition by advocating the Materials and Methods given below.

2. Materials and Methods

Efficacy of ten phytoextracts, six fungal and six bacterial biocontrol agents were evaluated against *A. alternata* under laboratory condition at Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh.

2.1 Efficacy of phytoextracts against *A. alternata in vitro*

The efficacy of naturally available ten phytoextracts with three concentrations viz., 5, 7.5 and 10 per cent were tested against *A. alternata in vitro* following Poisoned Food Technique of Bagchi and Das (1968) [3] using Potato Dextrose Agar as a germinating medium. An experiment was laid out with ten treatments repeated thrice. Completely Randomized Design with Factorial concept was used for analyzing the experimental data.

Fresh leaves, rhizomes, cloves or bulbs of ten locally available plant species were collected and thoroughly washed with running tap water followed by sterilized distilled water. Each sample was homogenized in sterilized distilled water at the rate of 1 ml/g of tissues (1:1 V/W) with a pestle and mortar and filtered through fine muslin cloth. The filtrate thus obtained was centrifuged at 5000 rpm for 10 minutes and the supernatant was filtered with the sterilized funnel having the pore size of 1-2 μ , which was consider standard plant extract solution (100%) and was stored in refrigerator at 4 °C for further use. Required concentrations of respective phytoextracts were prepared accordingly (Ansari, 1995) [2]. A measured quantity of plant extract was individually incorporated into measured volume of PDA medium without change in the composition at 5, 7.5 and 10 per cent concentration in 250 ml conical flasks to make final volume of 60 ml and later on, sterilized at 1.055 kg/cm² (15 psi) at 121.6 °C for 15 minutes in an autoclave. After that, this molten poisoned medium was poured at the rate of 20 ml per 90 mm (diameter) sterilized Petri plates and allowed to solidify. After solidification, an actively growing mycelial bit of test pathogen having 4 mm diameter cut with the help of sterilized cork-borer were transferred under aseptic condition in an inverted position in the center of the plate to make a direct contact with solidified PDA medium and were incubated at 28 $\pm$ 1 °C. Control plate was also maintained simultaneously without adding phytoextracts for each concentration till attains full growth and observations were recorded on linear mycelial growth in treated and control plates. The per cent mycelial growth inhibition of fungus in each treatment was calculated by using the formula given by

Vincent (1947) [18].

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

Where,

I = Per cent inhibition of mycelial growth

T = Mean radial growth of test pathogen in treated plate (mm)

C = Mean radial growth of test pathogen in control plate (mm)

2.2 Efficacy of fungal biocontrol agents against *A. alternata in vitro*

The antagonistic effect of six fungal biocontrol agents viz., *Trichoderma viride* JAU isolate, *T. harzianum* JAU isolate, *T. virens* JAU isolate, *T. koningii* JAU isolate, *T. hamatum* JAU isolate and *T. asperellum* JAU isolate each at (2 $\times$ 10⁶ cfu/g minimum) were tested against *A. alternata* using Dual Culture Technique of Morton and Stroube (1955) [12] in Completely Randomized Design with four repetitions. Twenty milliliters of sterilized melted potato dextrose agar (PDA) media were poured aseptically in each 90 mm diameter Petri plate and were allowed to solidify. After solidification, a mycelial disc of four millimeters diameter of each antagonist and test fungus were cut with the help of sterilized cork-borer from the edges of actively growing culture were placed aseptically on the PDA medium in the same Petri plates, on opposite corners by keeping one cm distance from distal ends of Petri plates. A control plate was also maintained separately by placing two pathogens in the same plate, on the opposite corner. The inoculated plates were incubated at 28 $\pm$ 1 °C in BOD incubator. The plates were observed periodically for the growth of the antagonist and test fungus.

2.3 Efficacy of bacterial biocontrol agents against *A. alternata in vitro*

The antagonistic effect of six bacterial biocontrol agents viz., *Pseudomonas fluorescens* JAU isolate-1, *P. fluorescens* JAU isolate-2, *Bacillus amyloliquefaciens* NAU isolate, *B. subtilis* JAU isolate-1, *B. subtilis* JAU isolate-2 and *Bacillus cereus* JAU isolate were tested against *A. alternata* with four repetitions. The test fungus was dual culture plated with different six bacterial antagonist as described by Montealegre *et al.* (2003) [11]. Twenty milliliters of sterilized melted PDA (50%) + NA (50%) media were poured aseptically in each 90 mm diameter Petri plate and were allowed to solidify. After solidification, a mycelial disc of four millimeters diameter of test fungus was cut with the help of a sterilized cork-borer from the edges of actively growing culture and were placed aseptically in the center of PDA (50%) + NA (50%) media containing Petri plates. A circular line made by dipping periphery of glass funnel of the size 60 mm diameter in a suspension of different bacterial antagonists (1 $\times$ 10⁸ cfu/ml minimum) surrounding the fungal inoculum. The inoculated plates were then incubated for 72 h at 28 $\pm$ 1 °C. A control plate was also maintained separately by placing pathogen in the centre of the Petri plate and the fungal growth was measured in all treatments and compared with control plate where the bacterial suspension replaced by sterile distilled water (Rupapara *et al.*, 2019) [15].

The radial growth of the test pathogen was measured after five days of inoculation. The per cent mycelial growth inhibition of the test fungus in each treatment by the fungal and bacterial biocontrol agents were calculated by using the

formula given earlier in 2.1.

2.4 Statistical analysis

Statistical analysis was carried out as per standard methods as suggested by Panse and Sukhatme (1985) [13]. Actual percentage data were converted to angular values and analyzed statistically.

3. Results and Discussion

The observations on mycelial growth inhibition were recorded after nine days of inoculation of phytoextracts and five days of inoculation of fungal and bacterial biocontrol agents. The results obtained are communicated here under.

3.1 Efficacy of phytoextracts against *A. alternata* in vitro

The data presented in Table 1, Figure 1 and Plate I indicated that all the phytoextracts were capable of reducing the mycelial growth of *A. alternata* in vitro. Among different phytoextracts tried, the maximum mean mycelial growth inhibition of (52.13%) was observed in rhizome extract of *Zingiber officinale* Rosc. which remained significantly superior over rest of the treatments. Whereas, leaf extract of *Ocimum sanctum* L. and leaf extract of *Datura stramonium* L. exhibited minimum mean mycelial growth inhibition of 19.25 and 21.47 per cent, respectively. While, looking to the interaction effect, rhizome extract of *Zingiber officinale* Rosc. at 10 per cent concentration showed the maximum mycelial growth inhibition (65.42%) and proved significantly superior over rest of the treatments. But, it was remained statistically at par with leaf extract of *Calotropis gigantea* L., bulb extract of *Allium cepa* L. and clove extract of *Allium sativum* L. at 10 per cent concentration with 64.68, 63.94 and 62.83 per cent mycelial growth inhibition, respectively. However, bulb extract of *Allium cepa* remained the less effective treatment as compared to other phytoextracts and gave minimum mycelial growth inhibition of 9.02 per cent at 5 per cent concentration followed by leaf extract of *Aegle marmelos* L. (12.57%) at 5 per cent concentration.

The results obtained in present study were corroborate with more or less with similar type of results as revealed by Choudhary (2024) [6]. He reported effectiveness of extract of ginger rhizome at 10 and 5 per cent concentration in inhibiting the mycelial growth of *A. alternata* (84.88 and 77.71%) causing Alternaria leaf blight of isabgol disease. Shekhada *et al.* (2023) [17] also revealed 40.74 per cent mycelial growth inhibition of *A. alternata* using onion bulb extract at 10 per cent concentration. Meena *et al.* (2022) [10] observed minimum inhibition of *A. alternata* of fennel by using tulsi extract (23.16%) which support the present findings. Antimicrobial activity of phytoextracts seems to be due to their direct toxicity and diverse antifungal properties of plant extracts as suggested by Zubrod *et al.* (2019) [20]. Ficker *et al.* (2003) [7] proposed the rhizome extract of *Zingiber officinale* (ginger) inhibits fungal mycelial growth due to the presence of bioactive compounds such as gingerols and zingerone. These phytochemicals disrupt cell membrane integrity, inhibit vital fungal enzymes and induce oxidative stress, leading to leakage of cellular contents and suppression of hyphal growth.

3.2 Efficacy of fungal biocontrol agents against *A. alternata* in vitro

The data presented in Table 2, Figure 2 and Plate II indicated

that all the antagonistic fungi were capable of reducing the mycelial growth of *A. alternata* in vitro. *Trichoderma harzianum* JAU isolate (2×10^6 cfu/g min.) gave maximum mycelial growth inhibition of 47.02 per cent and proved significantly superior over rest of the treatments. The next effective treatment in order of merit was *T. hamatum* JAU isolate with 40.66 per cent mycelial growth inhibition, but it was remained statistically at par with *T. koningii* JAU isolate with mycelial growth inhibition of 38.90 per cent. Whereas, *T. virens* JAU isolate remained moderately effective treatment with mycelial growth inhibition of 37.24 per cent and was at par with *T. asperellum* JAU isolate (36.34%). Whereas, *T. viride* JAU isolate showed the least effective with 14.06 per cent mycelial growth inhibition of *A. alternata*.

The present study was in line with the results obtained by Zade *et al.* (2018) [19]. They reported least growth of *A. alternata* of soybean in presence of *Trichoderma harzianum* (79.65%) in vitro. The effectiveness of *T. harzianum* (56.89%) followed by *T. koningii* (53.11%) and least with *T. virens* (49.11%) were also reported by Shamurailatpam and Kumar (2020) [16] against *A. solani* causing early blight of tomato.

An antimicrobial activity of fungal biocontrol agents seems to be due to competition for food, production of antimicrobial compounds and mycoparasitism of fungal biocontrol agents on *Alternaria* fungus that caused lysis of the spores and hyphae.

3.3 Efficacy of bacterial biocontrol agents against *A. alternata* in vitro

The data presented in Table 3, Figure 3 and Plate III indicated that all the bacterial biocontrol agents significantly inhibited the mycelial growth of *A. alternata* in vitro. Among different bacterial biocontrol agents, the maximum mycelial growth inhibition was observed in *Bacillus amyloliquefaciens* NAU isolate with 44.82 per cent and proved significantly superior over rest of the treatments, but it was found statistically at par with *Pseudomonas fluorescens* JAU isolate 1 (44.10%). The next effective treatment in order of merit was *Bacillus cereus* JAU isolate with 43.22 per cent mycelial growth inhibition. The treatment *Pseudomonas fluorescens* JAU isolate 2 found moderately effective with mycelial growth inhibition of 35.81 per cent and remained at par with *Bacillus subtilis* JAU isolate 2 (34.83%). Whereas, *Bacillus subtilis* JAU isolate 1 found least effective treatment with 27.40 per cent mycelial growth inhibition of *A. alternata*.

A more or less similar kinds of result was also reported by Bajaya (2017) [4]. He reported the effectiveness of *Pseudomonas fluorescens* (60.40%) and *Bacillus subtilis* (44.20%) in reducing mycelial growth of *A. alternata* in isabgol under in vitro condition. According to Khursheed *et al.* (2021) [8] *B. subtilis* (51.21%) remained the least effective in inhibition of mycelial growth of *A. alternata* which corroborate the present findings. The bacterial antagonists *Pseudomonas* spp. produces phenazines, phloroglucinols, dialkyl-resorcinols, pyoluteorin and pyrrolnitrin like compounds which may act as a mechanism of action in biological control (Chin-A-Woeng *et al.*, 2003) [5]. *Bacillus* spp. produce several bacteriocins with antimicrobial activity such as amylolysin, amylocyclicin, amysin, subtilin, subtilisin A, subtilisin B, thuricin (Abriouel *et al.*, 2011) [1] with antifungal activity.

Table 1: Efficacy of different phytoextracts against *A. alternata* in vitro

Phytoextracts	Mycelial growth inhibition (%)*			Mean mycelial growth inhibition (%)
	5%	7.5%	10%	
<i>Adhatoda vasica</i> Ness. (Ardusi)	25.08 (17.97)	38.92 (39.48)	46.09 (51.89)	36.70 (36.44)
<i>Allium cepa</i> L. (Onion)	17.48 (9.02)	46.83 (53.19)	53.09 (63.94)	39.13 (42.05)
<i>Allium sativum</i> L. (Garlic)	24.94 (17.79)	33.46 (30.40)	52.43 (62.83)	36.94 (37.00)
<i>Azadirachta indica</i> L. (Neem)	28.25 (22.40)	34.37 (31.87)	41.94 (44.67)	34.85 (32.98)
<i>Calotropis gigantea</i> L. (Aakdo)	24.66 (17.40)	33.68 (30.76)	53.53 (64.68)	37.29 (37.61)
<i>Zingiber officinale</i> Rosc. (Ginger)	32.63 (29.08)	51.88 (61.90)	53.98 (65.42)	46.17 (52.13)
<i>Datura stramonium</i> L. (<i>Datura</i>)	23.90 (16.42)	28.89 (23.34)	29.77 (24.65)	27.52 (21.47)
<i>Ocimum sanctum</i> L. (Tulsi)	22.02 (14.06)	23.66 (16.11)	31.68 (27.59)	25.79 (19.25)
<i>Vitex negundo</i> L. (Nagod)	24.23 (16.84)	25.91 (19.09)	38.38 (38.55)	29.50 (24.82)
<i>Aegle marmelos</i> L. (Bael)	20.77 (12.57)	26.96 (20.55)	42.58 (45.78)	30.10 (26.30)
Mean	22.18 (15.78)	31.32 (29.70)	40.32 (44.54)	-
	Phytoextract (P)		Concentration (C)	P x C
S. Em. $\pm$	0.40		0.22	0.70
C. D. at 5%	1.14		0.63	1.98
C.V.%	3.52			

Note: * Mean of three repetitions

Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

Table 2: Efficacy of different fungal biocontrol agents against *A. alternata* in vitro

Fungal biocontrol agents	Mycelial growth inhibition (%)*
<i>Trichoderma viride</i> JAU isolate	12.09 (14.06)
<i>Trichoderma harzianum</i> JAU isolate	45.04 (47.02)
<i>Trichoderma virens</i> JAU isolate	35.27 (37.24)
<i>Trichoderma koningii</i> JAU isolate	36.92 (38.90)
<i>Trichoderma hamatum</i> JAU isolate	38.69 (40.66)
<i>Trichoderma asperellum</i> JAU isolate	34.37 (36.34)
S. Em. $\pm$	0.60
C. D. at 5%	1.97
C. V.%	3.94

Note: * Mean of four repetitions

Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

Table 3: Efficacy of different bacterial biocontrol agents against *A. alternata* in vitro

Bacterial antagonists	Mycelial growth inhibition (%)*
<i>Pseudomonas fluorescens</i> JAU isolate 1	42.59 (44.10)
<i>Pseudomonas fluorescens</i> JAU isolate 2	34.30 (35.81)
<i>Bacillus amyloliquefaciens</i> NAU isolate	43.31 (44.82)
<i>Bacillus subtilis</i> JAU isolate 1	25.90 (27.40)
<i>Bacillus subtilis</i> JAU isolate 2	33.32 (34.83)
<i>Bacillus cereus</i> JAU isolate	41.72 (43.22)
S. Em. $\pm$	0.51
C. D. at 5%	1.50
C. V.%	2.74

Note: * Mean of four repetitions

Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

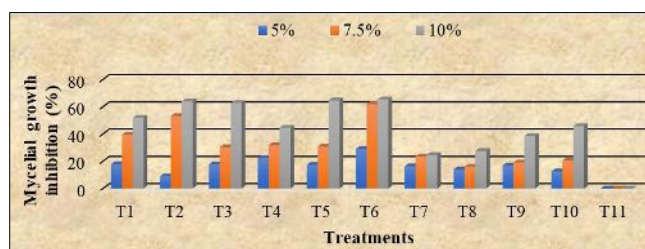
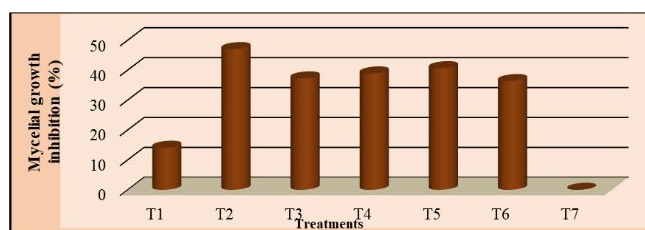
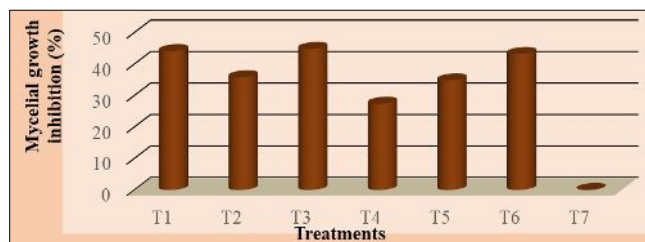
**Fig 1:** Efficacy of different phytoextracts against *A. alternata* in vitro**Fig 2:** Efficacy of different fungal biocontrol agents against *A. alternata* in vitro**Fig 3:** Efficacy of different bacterial biocontrol agents against *A. alternata* in vitro



Plate I: Efficacy of different phytoextracts against *A. alternata* in vitro

Phytoextracts

- T₁: *Adhatoda vasica* Ness. (Ardusi)
 T₂: *Allium cepa* L. (Onion)
 T₃: *Allium sativum* L. (Garlic)
 T₄: *Azadirachta indica* L. (Neem)
 T₅: *Calotropis gigantea* L. (Aakdo)
 T₆: *Zingiber officinale* Rosc. (Ginger)
 T₇: *Datura stramonium* L. (Datura)
 T₈: *Ocimum sanctum* L. (Tulsi)
 T₉: *Vitex negundo* L. (Nagod)
 T₁₀: *Aegle marmelos* L. (Bael)
 T₁₁: Control



Plate II: Efficacy of different fungal biocontrol agents against *A. alternata* in vitro

Fungal biocontrol agents

- T₁: *Trichoderma viride* JAU isolate
 T₂: *Trichoderma harzianum* JAU isolate
 T₃: *Trichoderma virens* JAU isolate
 T₄: *Trichoderma koningii* JAU isolate
 T₅: *Trichoderma hamatum* JAU isolate
 T₆: *Trichoderma asperellum* JAU isolate
 T₇: Control



Plate III: Efficacy of different bacterial biocontrol agents against *A. alternata* in vitro

Bacterial biocontrol agents

- T₁: *Pseudomonas fluorescens* JAU isolate-1
 T₂: *Pseudomonas fluorescens* JAU isolate-2
 T₃: *Bacillus amyloliquefaciens* NAU isolate
 T₄: *Bacillus subtilis* JAU isolate-1
 T₅: *Bacillus subtilis* JAU isolate-2
 T₆: *Bacillus cereus* JAU isolate
 T₇: Control

4. Conclusion

The present study revealed that all the phytoextracts as well as fungal and bacterial biocontrol agents were capable of reducing the mycelial growth of *Alternaria alternata* to different degrees under *in vitro* condition. Among different ten phytoextracts tested *in vitro*, the maximum mycelial growth inhibition was observed in rhizome extract of *Zingiber officinale* Rosc. (65.42%). But, it was remained statistically at par with leaf extract of *Calotropis gigantea* L.

(64.68%), bulb extract of *Allium cepa* L. (63.94%) and clove extract of *Allium sativum* L. (62.83%) each at 10 per cent concentration. Among different fungal biocontrol agents tested *in vitro*, *Trichoderma harzianum* JAU isolate (2×10^6 cfu/g min.) exhibited maximum antifungal activity against test pathogen *in vitro*. Whereas, among different bacterial biocontrol agents *Bacillus amyloliquefaciens* NAU isolate (1×10^8 cfu/ml min.) exhibited potential inhibitory action against test pathogen. But, it was remained statistically at par with *Pseudomonas fluorescens* JAU isolate 1 (1×10^8 cfu/ml min.).

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