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RESEARCH ARTICLE

Consortium of biocontrol agents, plant oils and oil cakes for the management of leaf blight of jasmine caused by *Alternaria jasmini*

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Abstract

Chemical pesticides are widely used for plant disease management, but their negative impacts on human health and the environment necessitate the search for safer alternatives. Essential oils and oil cakes have shown promising potential as eco-friendly and sustainable solutions for plant disease control. This study aimed to assess the effectiveness of bacterial antagonists, plant-based oils and oil cakes in managing jasmine leaf blight caused by *Alternaria jasmini*. Among the seven bacterial isolates tested, *Pseudomonas indica* (AUPP23) exhibited the highest inhibitory activity, reducing mycelial growth by 74.70 %. Tulasi oil (1 %) was the most effective plant oil, completely inhibiting the growth of the pathogen. Neem cake extract at 20 % concentration resulted in the highest reduction in mycelial growth of the pathogen, with a suppression rate of 97.41 %. Consortium of foliar application with *P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + tulasi oil (1 %) + neem cake extract (10 %) had the highest PDI of 09.97 % with 87.32 % disease reduction over control, which was comparable to that of mancozeb (0.2 %), which had a PDI of 09.02 % with 88.53 % disease reduction over control. Furthermore, the highest flower output of 114.27 kg/15 cents was reported with the same treatment. These findings indicate that a consortium of plant-derived oils, such as tulasi oil, along with bacterial bioagents and neem cake extract serves as an effective bio-fungicide alternative. In addition to improving flower output, this environmentally benign method effectively manages jasmine leaf blight disease.

Keywords: *Alternaria jasmini; Bacillus subtilis;* jasmine; neem cake; *Pseudomonas indica;* tulasi oil

Introduction

In India, jasmine (*Jasminum sambac* L. Aiton), a genus of the Oleaceae family, is a major traditional flower crop, widely cultivated in Tamil Nadu for its high commercial value as a loose flower. It was introduced in the mid-sixteenth century, which is a native to tropical and subtropical regions. The most cultivated jasmine species are *Jasminum sambac* (Gundu mallige) and *Jasminum grandiflorum* (Jaaji mallige), which are primarily grown for the fresh flower market, while *Jasminum auriculatum* (Sooji mallige) is mainly cultivated for concrete extraction. Jasmine is grown extensively across India, with Tamil Nadu being the leading producer, particularly in regions like Coimbatore, Madurai and Dindigul. The state has an annual production of 180.67 tons from 13726 hectares of cultivated area (1). In addition, Karnataka (Kolar, Bangalore, Bellary and Mysore), Uttar Pradesh (Kannuaj, Jaunpur and Gazipur), Rajasthan (Ajmer and Kota), West Bengal (Ranaghat, Kolaghat and Pancskura) and portions of Andhra Pradesh and Maharashtra are important producers. The jasmine flowers from Tamil Nadu are exported to neighboring countries like Sri Lanka, Singapore, Malaysia and various Middle East countries.

However, jasmine plants are extremely vulnerable to a variety of diseases brought on by bacterial, viral and fungal infections, which severely reduces production and financial gains. Leaf spot, a common fungal disease brought on by *Alternaria jasmini*, is a serious hazard to jasmine crops. The pathogen primarily infects plants under dry, warm conditions and is disseminated through airborne spores, with peak disease incidence observed during the rainy season. An *Alternaria jasmini* infection results in dark, necrotic patches with concentric rings forming at the leaf tips. These spots spread quickly during the rainy season. In extreme situations, new shoots may also desiccate and afflicted leaves may curl and dry from the edges. This infection leads to a drastic reduction in flower production, potentially causing up to a 50 % yield loss (2). For ornamental growers, maintaining plant health is a critical concern, as market demand necessitates flawless flowers, leaves, stems and roots. The economic

tolerance for pest and disease damage is exceptionally low (3), emphasizing the need for effective plant health management strategies.

Although drugs are typically used to manage many diseases, there is growing interest in using alternate strategies including biological control agents (4). Agricultural researchers are searching for environmentally friendly, ecologically sustainable and medically safe alternatives that specifically target plant pathogens due to concerns about the harmful effects of chemical pesticides, including contamination of agricultural land, water and soil, as well as related health risks (5). The uses of biological control agent to control diseases in flower crops is receiving more attention (4). As biocontrol techniques, microbial antifungal agents provide a possible substitute. High specificity against target pathogens, environmental degradability and economical mass manufacturing is some of the attractive properties of these microorganism derived materials (6). In a variety of crops, it has been shown that antagonistic bacteria such as *Pseudomonas* and *Bacillus* (7), plant-based oils (8, 9) and oil cake extracts (9) are effective in controlling *Alternaria* spp. Considering this, the current study aims to investigate ecologically friendly methods for the efficient management of *A. jasmini*.

Materials and Methods

The *in vitro* study has conducted during 2021–2024 at the Department of Plant Pathology, Annamalai University, Chidambaram, Tamil Nadu (11°39'18" N, 79°72'32" E) at elevation of 5.79 m above sea level. The region experiences a hot and humid climate with an average annual rainfall of 1351.4 mm, 70 % of which occurs during October-November. The soil is clay loam, well-drained and of medium fertility.

Crop : Jasmine (*Jasminum sambac* L. Aiton)

Cultivar : Ramnad kundu malli

Survey

A survey was conducted in various districts of Tamil Nadu, including Cuddalore (B. Mutlur, Vallampadugai, Theethampalayam), Madurai (Melur), Salem (Morepalayam), Sivagangai (Melakadu) and Thiruvarur (Salur), during 2021 to evaluate the intensity of leaf blight disease in jasmine. In each location, three fields were selected at random. From each field, 100 leaves were randomly sampled and percent disease index (PDI) was assessed as per the standard grade chart given below.

Grades	Leaf area affected (per cent)
0	Healthy
1	1-5
3	6-10
5	11-25
7	26-50
9	More than 50

PDI has been worked out by Mckinney formula (10) *viz.,*

$$\text{Percent disease index} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}} \quad \text{(Eq. 1)}$$

Isolation of pathogen

The leaf blight pathogen, *Alternaria jasmini* (Fig. 1), was isolated using the tissue segment method (11). Freshly infected leaves exhibiting characteristic symptoms were collected and the lesion margins were carefully excised into small segments using a sterilized scalpel. The collected tissue samples were disinfected by immersing them in 0.1 % mercuric chloride for 1 min, followed by three washes with sterile distilled water. The sterilized samples were then placed on Potato Dextrose Agar (PDA) medium in Petri dishes and incubated at 28 ± 2 °C for five days to encourage fungal growth. Hyphal growth emerging from the tissue pieces was carefully transferred to PDA slants under aseptic conditions for culture preservation. The pathogen was identified based on its morphological and cultural traits, following the descriptions provided by Simmons (12) (Fig. 2, 3). A total of seven isolates, designated as I1 to I7, were obtained. Among them, the most virulent isolate, I1, as determined through pathogenicity tests, was selected for further investigations.

Isolation of bacterial antagonists from jasmine phylloplane microflora

Jasmine leaves were gathered from various jasmine-cultivating regions and carefully cut into small sections using the sterile scalpel. Trimmed leaves fragment were then submerged in 10 mL of distilled water, vigorously agitated for 5 min and subsequently left undisturbed for another 5 min. For bacterial isolation, King's B medium was employed for *Pseudomonas* spp., while nutrient agar was used for other bacterial strains. Each Petri plate was filled with 20 mL of the respective medium, gently swirled for uniform distribution and allowed to solidify. 1 mL of the prepared suspension was then aseptically transferred into sterilized Petri plates using a sterile pipette. The plates were incubated at room temperature (28 ± 2 °C) for 48 hr. Post-incubation, bacterial colonies were sub-cultured and purified using the streak plate method, as outlined by Rangaswami and Sowmini Rajagopalan (13). This process led to the successful isolation of seven *Pseudomonas* spp. (BPf, TPf, AUPP23, MEPf, MKPf, MOPf and SAPf) and seven *Bacillus* spp. (Bs1, Bs2, Bs3, Bs4, Bs5, Bs6 and Bs7) from the phyllosphere of jasmine plants.

In vitro assessment of phylloplane bacterial antagonists against *Alternaria jasmini*

Seven isolates of *Pseudomonas indica* and *Bacillus subtilis* obtained from jasmine-growing regions of Tamil Nadu were

Fig. 1. Symptoms of jasmine leaf blight caused by *A. jasmini.* A) Infected leaf B) Healthy leaf.

tested for their antagonistic potential against *Alternaria jasmini* using the dual culture method (14). A 9 mm disc of actively growing *A. jasmini* culture was carefully placed on one side of a PDA plate, positioned 1.5 cm from the edge. On the opposite side of the plate, approximately 1.5 cm from the edge, a 1 cm streak of a two-day-old bacterial culture (*P. indica* and *B. subtilis*) was carefully inoculated onto the medium. To serve as a control, *A. jasmini* was inoculated alone at one end of a Petri plate. The plates were then incubated at 28 ± 2 °C under standard laboratory conditions, with each treatment repeated three times. Once the fungal growth in control plate had fully developed, the radial growth of *A. jasmini* in the treated plates was recorded. The percentage of growth inhibition in comparison to the control was calculated using the formula (15).

$$\text{Percent of inhibition} = \frac{C-T}{C} \times 100 \qquad \text{(Eq. 2)}$$

Where,

C = Diameter of the pathogen in control

T = Diameter of the pathogen in treatment

Efficacy of plant oils against *Alternaria jasmini in vitro*

The antifungal potential of 15 plant derived oils including almond, castor, citriodora, citronella, coconut, groundnut, lemongrass, mahua, mustard, neem, olive, pungam, sesame, sunflower and tulasi oils was assessed against *Alternaria jasmini* using the poisoned food technique (16). The oils were tested at concentrations ranging from 0.6 % to 1.0 %. To conduct the assay, PDA medium was prepared, sterilized at 121 °C for 15 min and allowed to cool. The required quantity of each oil was thoroughly incorporated into the sterilized PDA before pouring 20 mL of the oil infused medium into sterile Petri plates, which were then left to solidify. A 9 mm mycelial disc from an actively growing virulent isolate of *A. jasmini* was carefully placed at the centre of each plate. PDA without any oil served as the control. All procedures were performed under aseptic conditions and each treatment was replicated three times. The plates are maintained at ambient temperature (28 ± 2 °C) for incubation. Once fungal growth in control plates reached its maximum, the radial mycelial growth was recorded for all treatments and the percentage of pathogen inhibition compared with control was calculated.

Efficacy of oil cake extracts against *Alternaria jasmini in vitro*

The antagonistic potential of oil cake extracts against *A. jasmini* was assessed using the poisoned food technique (16). Freshly prepared PDA medium (100 mL) was dispersed into multiple conical flasks. Aqueous extracts of oil cakes were added to the PDA medium at concentrations of 10 %, 15 % and 20 % by mixing 10 mL, 15 mL and 20 mL of the extract with 100 mL of PDA respectively. The prepared media were sterilized and poured (15 mL per plate) into sterilized Petri dishes, where they were allowed to solidify. The plates were incubated at room temperature with a 9 mm mycelial disc from an *A. jasmini* culture that was actively developing in the middle of each dish. Control plates containing PDA without oil cake extracts were also prepared. The fungal pathogen's radial expansion was recorded once the control plates exhibited complete growth. The results were recorded in millimetres and used to calculate the percentage inhibition of the pathogen.

Compatibility test

The compatibility between the bacterial biocontrol agents *P. indica* (AUPP23) and *B. subtilis* (Bs7) was evaluated using the method described by earlier researchers (17). The test involved streaking the bacterial cultures horizontally and vertically on

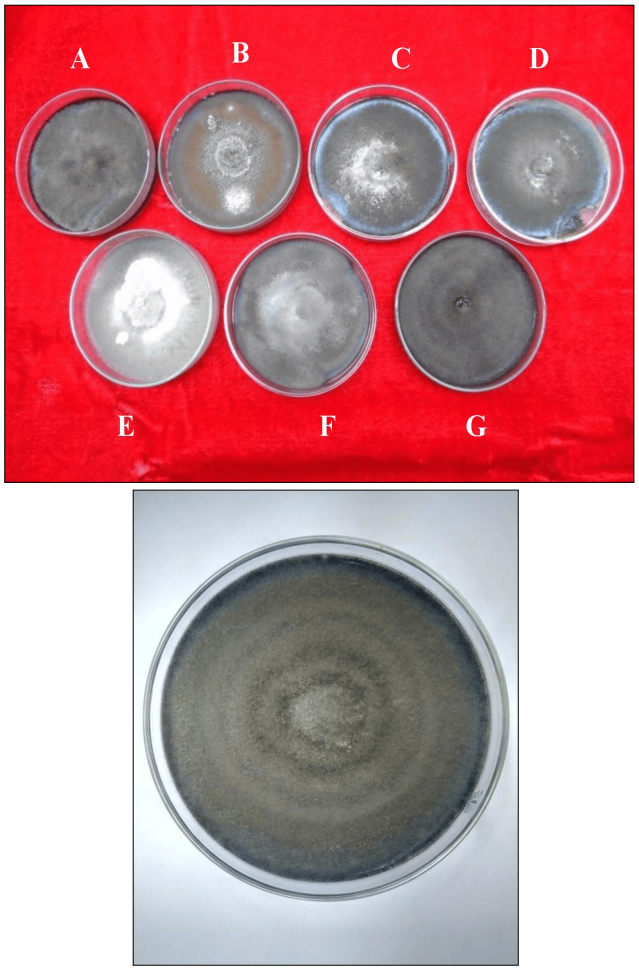
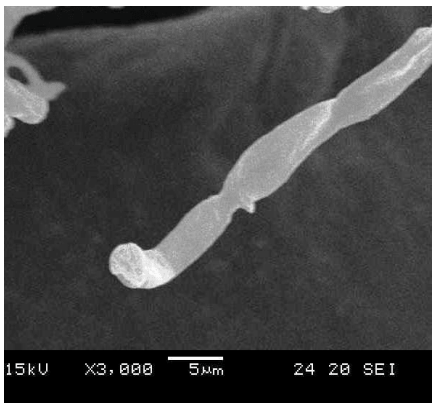


Fig. 2. Pure culture of *A. jasmini.* A) I1 B)I2 C)I3 D)I4 E)I5 F)I6 G)I7.



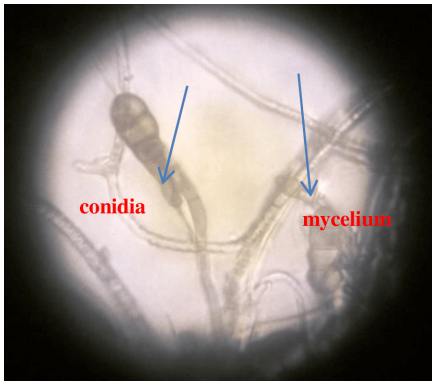


Fig. 3. SEM and microscopic observation of *A. jasmini.*

nutrient agar plates and maintain the control plates for each bacterial culture. The biocontrol agents were streaked onto medium supplemented with 10 % neem cake extract and 1 % tulasi oil separately. Control plates without amendments were also prepared for comparison. All plates were incubated at 28 ± 2 °C and monitored for the presence of inhibition zones. The absence of a zone of inhibition indicated compatibility, while the presence of a zone signified incompatibility.

Assessment of antagonistic microorganisms, oil cake extracts and plant oils for controlling jasmine leaf blight in pot culture

A pot culture experiment has been conducted during 2021-2024 to evaluate the effectiveness of antagonistic microorganisms, tulasi oil and neem cake extracts in managing jasmine leaf blight caused by *A. jasmini* under pot culture condition. This study has conducted with 12 treatments and 3 replications, using a local jasmine variety as the test crop. The leaves of jasmine plants were pinpricked with a sterile needle and a spore suspension of *A. jasmini* (5×10^5 spores/mL) was sprayed at 45th day after planting. Foliar applications of the treatments were performed on the 60th and 75th days after planting. To facilitate disease establishment, the plants were covered with the polythene bags and sprayed with sterile distilled water, maintaining water congestion for 24 hr before and after inoculation. Disease intensity was assessed 10 days after the second spray using a 0-9 scoring chart. The PDI was determined using McKinney's formula (10) and the results were presented as the percentage of disease reduction compared to the control.

The following were the treatments

T_1: *Pseudomonas indica* (AUPP23) (0.2 %)

T_2: *Bacillus subtilis* (Bs7) (0.2 %)

T_3: T_1 + T_2

T_4: T_1 **+** Tulasi oil (1 %)

T_5: T_2 + Tulasi oil (1 %)

T_6: T_3 + Tulasi oil (1 %)

T_7: T_1 + Neem cake extract (10 %)

T_8: T_2 + Neem cake extract (10 %)

T_9: T_3 + Neem cake extract (10 %)

T_{10}: T_6 + Neem cake extract (10 %)

T_{11}: Mancozeb (0.2 %)

T_{12}: Pathogen-inoculated control

Biological management of jasmine leaf blight under the field conditions

In 2023, a randomized block design (RBD) with 8 treatments and 3 replications was used in a field study at B. Mutlur village, Chidambaram block, Cuddalore district. The trial was established in an existing jasmine field already affected by leaf blight. The foliar application of effective antagonistic microorganisms, neem cake extract and tulasi oil, was tested against jasmine leaf blight caused by *A. jasmini*. The effective treatments tested under pot culture conditions were evaluated in the farmer's field using the local variety.

At 15 days following the third spraying, the disease intensity was measured using the previously reported 0-9 grade score scale. The PDI was calculated using McKinney's formula (10) and the percentage reduction in disease intensity over the control was determined. Additionally, the yield of jasmine was measured. Nine jasmine shrubs were assessed for each treatment and flower buds were collected from the fixed plots on the 60th and 75th days after spraying. The pooled data of each was considered for the calculation of yield of jasmine. The following were the treatments.

The following were the treatments

T1: *Pseudomonas indica* (AUPP23) (0.2 %)

T2: *Bacillus subtilis* (Bs7) (0.2 %)

T3: T_1 + T_2

T4: T_3 + Tulasi oil (1 %)

T5: T_3 + Neem cake extract (10 %)

T6: T_4 + Neem cake extract (10 %)

T7: Mancozeb (0.2 %)

T8: Control

Statistical analysis

The Indian Council of Agricultural Research, Goa's Wasp version 2.0 was used to statistically analyze the data (18). The percentage values of the disease index were arcsine-transformed prior to statistical analysis. Analysis of variance (ANOVA) was performed at two significant levels ($P < 0.05$ and $P < 0.01$) and Duncan's Multiple Range Test (DMRT) was used to compare the means. Laboratory experiments, pot culture and field experiments were conducted using RBD. Arcsine or square root transformations were used to change the percentage numbers.

Results and Discussion

Survey and collection of isolates

The survey on the intensity of leaf blight of jasmine from different districts of Tamil Nadu ranged from 22.00 to 32.02 %. The results shown in Table 1 clearly reveal that the highest disease intensity, recorded at 32.02 %, was observed in B. Mutlur of Cuddalore district, followed by Melakadu (30.37 %) and Theethampalayam (27.12 %). The lowest disease intensity of 22.00 % was observed in Morepalayam village of Salem district (Table 1).

Similar findings were reported in an earlier study, where a survey on jasmine leaf blight in different jasmine-growing areas of Tamil Nadu recorded a disease index ranging from 25.46-61.22 % (8). The highest disease index of 61.22 % was recorded at Nilakottai in Dindigul district (19). The variation in disease index caused by *A. jasmini* may be attributed to the interaction between the pathogen and environmental factors prevailing in each locality (20).

Effectiveness of *Pseudomonas* spp. on mycelial growth of *Alternaria jasmini*

All tested isolates of *Pseudomonas* spp. demonstrated a significant inhibitory effect on the mycelial growth of *Alternaria jasmini* (Table 2). Among seven isolates evaluated, AUPP23 exhibited the highest inhibitory activity, restricting mycelial growth to 22.60 mm and achieving 74.70 % inhibition. This was followed by SAPf and MOPf, which recorded mycelial growth of 24.72 mm and 27.22 mm with inhibition rates of 72.33 % and

Table 1. Survey on the incidence of leaf blight of jasmine from different districts of Tamil Nadu

S. No.	Location	District	Soil type	Variety	PDI	Symptoms
1.	B. Mutlur	Cuddalore	Clay loam	Local	32.02[a] (34.46)	Dark brown/black spots with concentric ring and marginal blight
2.	Theethampalayam	Cuddalore	Clay	Local	27.12[c] (31.38)	Marginal blight with concentric ring
3.	Vallampadugai	Cuddalore	Clay loam	Iruvatchi	24.46[e] (29.63)	Brown spots surrounded by yellow halo
4.	Melur	Madurai	Black cotton soil	Ramanathapuram gundumalli	23.00[f] (28.65)	Marginal blight with concentric ring
5.	Melakadu	Sivaganga	Red soil	Ramanathapuram gundumalli	30.37[b] (33.44)	Brown spots leading to marginal blight
6.	Morepalayam	Salem	Red soil	Ramanathapuram gundumalli	22.00[g] (27.97)	Brown spots with concentric rings
7.	Salur	Thiruvarur	Black cotton soil	Ramanathapuram gundumalli	26.00[d] (30.65)	Marginal blight with concentric ring
		CD (P = 0.05 %)			**1.199**	

PDI: Percent disease index; *Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5 % level by DMRT; values in the parentheses represent arc-sine transformed values.

Table 2. Effect of Pseudomonas spp. on the mycelial growth of A. jasmini (I1)

S. No.	Treatments	Mycelial growth (mm)	Percentage inhibition over control
1.	*Pseudomonas* spp. (BPf)	34.21[f]	61.70
2.	*Pseudomonas* spp. (TPf)	31.67[de]	64.55
3.	*P. indica* (AUPP23)	22.60[a]	74.70
4.	*Pseudomonas* spp. (MRPf)	29.23[cd]	67.28
5.	*Pseudomonas* spp. (MKPf)	34.13[ef]	61.79
6.	*Pseudomonas* spp. (MOPf)	27.22[bc]	69.53
7.	*Pseudomonas* spp. (SAPf)	24.72[ab]	72.33
8.	Control	89.33[g]	-
	CD (P = 0.05 %)	**1.626**	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5 % level by DMRT.

69.53 % respectively. These isolates were statistically distinct from each other. The least inhibition (61.70 %) was observed with the BPf isolate, which recorded mycelial growth of 34.21 mm (Fig. 4). The biocontrol effectiveness of *Pseudomonas* spp. is linked to its ability to produce broad-spectrum antibiotics (21). Studies indicate that *Pseudomonas fluorescens* isolates can achieve high inhibition rates, reporting up to 77.73% inhibition against *Alternaria* spp. (22). These include phenazine from *Pseudomonas* sp. B109 in tomato (23, 24), 2,4-diacetylphloroglucinol (2,4-DAPG) from *Pseudomonas* sp. 28r/96 in wheat (25), pyoluteorin from *Pseudomonas* CHAO in tobacco (26), pyrrolnitrin from *Pseudomonas indica* BL915 in cotton (27) and viscosinamide from *Pseudomonas fluorescens* D1254 in sugar beet (28). These mechanisms, along with the production of siderophores, antibiotics and lipopolysaccharides, as noted in previous research (29), play a pivotal role in enhancing the antagonistic potential of *Pseudomonas* spp.

Effectiveness of *Bacillus* spp. on mycelial growth of *Alternaria jasmini*

The effectiveness of *Bacillus* spp. isolates against the mycelial growth of *A. jasmini* is summarized in Table 3. Among the seven isolates tested, Bs7 exhibited the highest inhibition, with a mean mycelial growth of 30.00 mm, corresponding to a 66.04 % reduction in growth. This was followed by Bs1 and Bs6, with mean growth of 31.35 mm (64.51 % inhibition) and 33.00 mm (62.64 % inhibition) respectively, showing statistically significant differences. The lowest inhibition rate (51.00 %) was recorded for MRBs, allowing mycelial growth of 43.30 mm (Fig. 5). The effectiveness of *Bacillus* spp. isolates in inhibiting the mycelial growth of *A. jasmini* is presented in Table 3. Among the seven isolates tested, Bs7 demonstrated the highest inhibitory effect, reducing mycelial growth to 30.00 mm, which corresponds to a 66.04 % inhibition rate. This was followed by Bs1 and Bs6 which recorded the mean mycelial growth of 31.35 mm (64.51 % inhibition) and 33.00 mm (62.64 % inhibition) respectively. These differences were statistically significant. Conversely, the lowest inhibition rate (51.00 %) was observed with MRBs, allowing mycelial growth of 43.30 mm (Fig. 5). Studies have demonstrated that specific *Bacillus* strains, such as *B. amyloliquefaciens* and *B. subtilis*, produce antimicrobial compounds like lipopeptides and cyclic lipopeptides, which disrupt the mycelial structure and membrane integrity of *Alternaria* spp., thereby inhibiting their growth and reducing toxin production (30, 31). The biocontrol activity of *B. subtilis* is attributed to its ability to produce a variety of antimicrobial peptides, such that subtilin, iturin, bacilysin and mycobacillin (32). Previous studies reported that fungal mycelial malformation might result from bacterial antibiotic metabolites, which penetrate the fungal structure, causing protoplasmic dissolution and disintegration (33). These findings align with the results of the current study, emphasizing the potential of *B. subtilis* as a biocontrol agent against *A. jasmini*.

Table 3. Effect of *Bacillus* spp. on the mycelial growth of *A. jasmini* (I1)

S. No	Treatments	Mycelial growth (mm)	Percentage inhibition over control
1.	*Bacillus* spp. (Bs1)	31.35[ab]	64.51
2.	*Bacillus* spp. (Bs2)	36.20[c]	59.02
3.	*Bacillus* spp. (Bs3)	43.30[e]	51.00
4.	*Bacillus* spp. (Bs4)	39.66[d]	55.10
5.	*Bacillus* spp. (Bs5)	42.13[de]	52.30
6.	*Bacillus* spp. (Bs6)	33.00[b]	62.64
7.	*Bacillus subtilis* (Bs7)	30.00[a]	66.04
8.	Control (pathogen alone)	88.33[f]	-
	CD (P = 0.05 %)	**1.846**	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

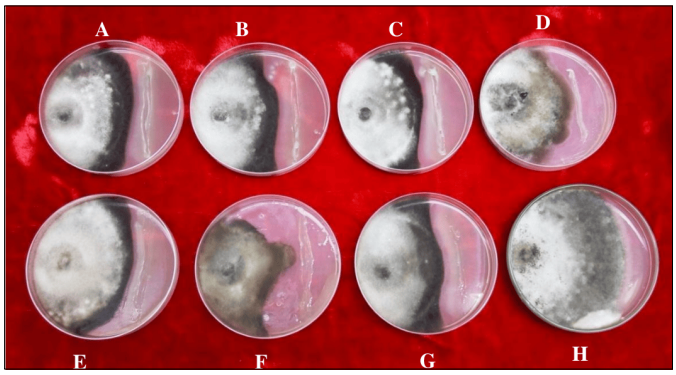


Fig. 4. Effect of *Pseudomonas* spp. on the mycelial growth of *A. jasmini* (I1). (A) BPf (B) TPf (C) AUPP23 (D) MEPf (E) MKPf (F) MOPf (G) SAPf (H) Control.

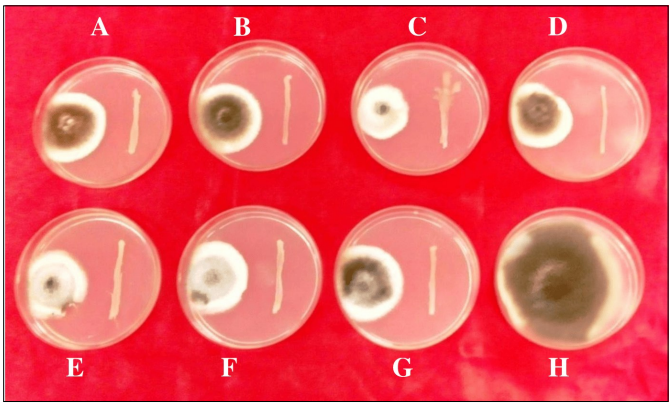


Fig. 5. Effect of *Bacillus* spp. on the mycelial growth of *A. jasmini* (I1). (A) Bs1 (B) Bs2 (C) Bs3 (D) Bs4 (E) Bs5 (F) Bs6 (G) Bs7 (H) Control.

In vitro assay of different plant oils against *Alternaria jasmini*

Among the various plant oils evaluated for their antifungal activity against *A. jasmini*, tulasi oil (1 %) demonstrated the highest efficacy, achieving complete (100 %) inhibition of mycelial growth. This was followed by citriodora oil, citronella oil and lemongrass oil. In contrast, sunflower oil (1 %) exhibited the least inhibitory effect, recording a mycelial growth of 57.17 mm with 35.76 % inhibition (Table 4; Fig. 6). Similarly, previous studies reported that among six plant oils tested against *A. solani*, *Ocimum basilicum* and *Ocimum sanctum* exhibited complete inhibition (100 %) of fungal growth across all tested concentrations (34). The study also indicated that oil efficacy was concentration-dependent, while microbial sensitivity varied among different oils (35, 36). Researchers identified methyl eugenol (82.9 %) as the predominant compound in tulasi oil through GC-MS analysis (37). Additional minor components included β-caryophyllene (4.1 %), α-copaene (1.9 %), germacrene D (2.3 %) and borneol (2.4 %). The major chemical groups identified were phenyl derivatives (83.8 %) followed by oxygenated monoterpenes (3.1 %), oxygenated sesquiterpenes (0.3 %) sesquiterpene hydrocarbons (11.1 %) and monoterpene hydrocarbons (0.6 %). The existence of these biologically active compounds in tulasi oil is likely responsible for its potent antifungal activity against *A. jasmini*.

In vitro assay of oil cake extracts against *Alternaria jasmini*

Overall, all organic amendments demonstrated a significant reduce the mycelial growth of the virulent pathogen. Among the eight amendments tested, neem cake extracts at a 20 % concentration exhibited the highest efficacy, recording the lowest mycelial growth of 2.32 mm and the maximum inhibition of 97.41 % compared to the control (Table 5). This was followed by sesame cake extract at the same concentration, which resulted in mycelial growth of 5.98 mm and a 93.33 % reduction over control (Fig. 7). Similarly, researchers evaluated the impact of four different oil cakes on the growth of *A. solani* (38). Among them, neem cake extract exhibited the highest inhibition (51.51 %) at a 30 % concentration after 120 hr of incubation, followed by mustard (44.56 %) and cotton (42.75 %). Neem contains a variety of bioactive compounds, including tetranorterpenoid lactones such as azadirachtin, nimbin, nimbidin, salanin and nimbolin B, with azadirachtin being the most potent antifeedant. Additionally, neem seed oil contains other bioactive constituents, including nimbolides, olichinolide B and azadiradione. Neem leaves also contain flavonoids like nimatone, quercetin, myricetin and kaempferol along with azadirachtin, meliantrol, salanin, β-sitosterol and stigmasterol (39). Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, alkaloids and saponins in neem leaves. These compounds, either individually or in synergy, may

Table 4. Efficacy of different plant oils on the mycelial growth of *A. jasmini* (I1)

S. No.	Treatments	Concentrations (%)									
		0.6		0.7		0.8		0.9		1.0	
		Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control
1.	Almond	69.28^{e}	21.85	54.92^{f}	37.82	43.55^{f}	51.06	35.76^{f}	59.97	25.79^{i}	71.02
2.	Castor	52.98^{cd}	40.24	38.89^{e}	55.97	28.46^{d}	68.02	19.33^{e}	78.36	15.91^{g}	82.12
3.	Citriodora	46.82^{a}	47.19	31.76^{ab}	64.04	24.69^{ab}	72.26	11.19^{b}	87.47	1.64^{b}	98.15
4.	Citronella	46.74^{a}	47.28	30.45^{a}	65.53	24.54^{ab}	72.43	11.14^{b}	87.53	1.46^{b}	98.35
5.	Coconut	49.09^{ab}	44.63	33.87^{bc}	61.65	27.51^{cd}	69.90	16.03^{c}	82.05	11.65^{e}	86.91
6.	Groundnut	51.20^{bc}	42.25	34.71^{cd}	60.70	26.98^{cd}	69.68	16.43^{c}	81.61	14.81^{f}	83.36
7.	Lemongrass	46.79^{a}	47.22	31.57^{ab}	64.26	24.61^{ab}	72.35	11.14^{b}	87.52	1.37^{ab}	98.46
8.	Mahua	48.95^{ab}	44.79	33.55^{bc}	62.02	25.89^{bc}	70.91	15.66^{c}	82.47	9.13^{d}	89.74
9.	Mustard	52.71^{cd}	40.55	35.31^{cd}	60.02	27.33^{cd}	69.29	17.72^{d}	80.16	15.23^{fg}	82.88
10.	Neem	47.81^{a}	46.07	32.17^{ab}	63.58	24.73^{ab}	72.21	11.21^{b}	87.45	2.10^{c}	97.64
11.	Olive	71.32^{e}	19.55	61.39^{g}	30.50	51.43^{g}	42.21	47.33^{g}	47.02	39.66^{j}	55.44
12.	Pungam	54.82^{d}	38.17	36.55^{d}	58.62	32.78^{e}	63.16	19.75^{e}	77.89	16.72^{h}	81.21
13.	Sesame	48.78^{ab}	44.98	32.11^{ab}	63.35	25.76^{bc}	71.06	11.27^{b}	87.38	2.15^{c}	97.58
14.	Sunflower	78.80^{f}	11.12	73.42^{h}	16.88	69.66^{h}	21.73	63.39^{h}	29.04	57.17^{k}	35.76
15.	Tulasi	46.71^{a}	47.32	30.26^{a}	65.74	23.47^{a}	73.63	10.23^{a}	88.55	0.00^{a}	100.00
16.	Control	88.66^{g}	-	88.33^{i}	-	89.00^{i}	-	89.33^{i}	-	89.00^{l}	-
CD (P = 0.05 %)		**2.690**	**-**	**2.308**	**-**	**1.902**	**-**	**0.875**	**-**	**0.807**	**-**

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5 % level by DMRT.

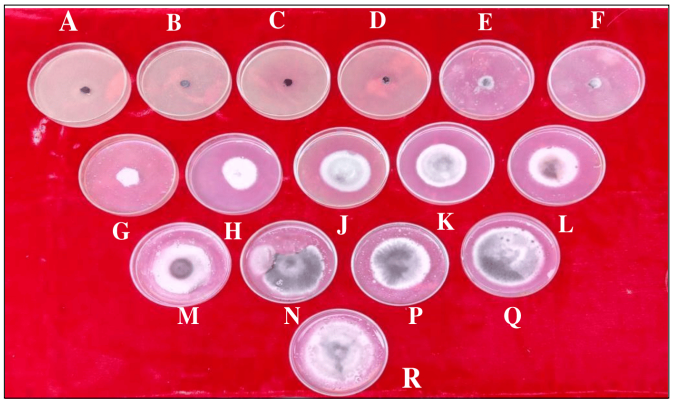


Fig. 6. Efficacy of different plant oils on the mycelial growth of A. jasmini (I1). (A) Tulasi (B) Citronella (C) Lemon grass (D) Citriodora (E) Neem (F) Sesame (G) Mahua (H) Coconut (J) Groundnut (K) Mustard (L) Castor (M) Pungam (N) Almond (P) Olive (Q) Sunflower (R) Control.

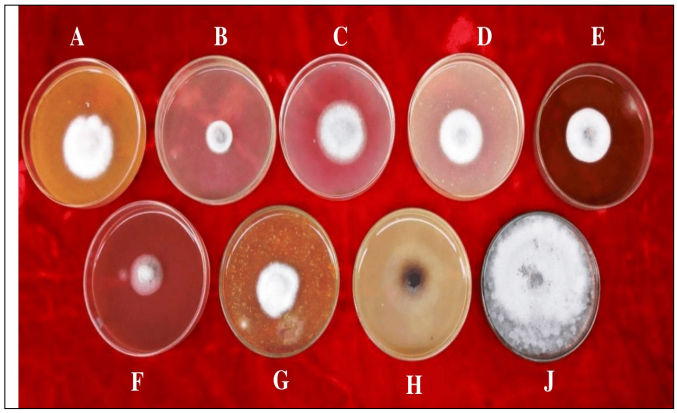


Fig. 7. Efficacy of different oil cakes on the mycelial growth of *A. jasmini* (I1). (A) Pungam (B) Sesame (C) Cotton (D) Coconut (E) Mahua (F) Neem (G) Groundnut (H) Castor (J) Control.

contribute to the suppression of *A. jasmini*. Researchers identified methyl eugenol (82.9 %) as the primary component in tulasi oil through GC-MS analysis (37). The predominant chemical group was phenyl derivatives (83.8 %), followed by oxygenated sesquiterpenes (0.3 %), sesquiterpene hydrocarbons (11.1 %), oxygenated monoterpenes (3.1 %) and monoterpene hydrocarbons (0.6 %). The presence of these bioactive compounds in plant oils is likely responsible for their inhibitory effect on *A. jasmini*.

Compatibility test

A study demonstrated that *P. indica* (AUPP23) and *B. subtilis* (Bs7) are compatible with each other, whereas *P. indica* (AUPP23) and *B. subtilis* (Bs7) are compatible with neem cake extract (10 %) and tulasi oil (1 %). This synergy highlights the potential for combining microbial biocontrol agents with botanical products to enhance efficacy against *A. jasmini*.

Evaluation of antagonistic microorganisms, oil cake extracts and plant oils for managing jasmine leaf blight under pot culture conditions

The effectiveness of post inoculation treatments involving antagonistic microorganisms, plant oils and oil cake extracts against jasmine leaf blight incidence is presented in Table 6. Among the different treatments evaluated, T_{10} comprising a foliar application of *P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + tulasi oil (1 %) + neem cake extract (10 %) proved to be the most effective, achieving a PDI of 13.55 % with an 81.35 % reduction in disease severity compared to the control. These results were comparable to the chemical fungicide mancozeb (0.2 %), which recorded a PDI of 13.00 % with an 82.11 % disease reduction. Following closely, treatment T_6 comprising a foliar spray of *P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + tulasi oil (1 %) resulted in a PDI of 15.64 %, corresponding to a 78.48 % disease reduction (Fig. 8). These findings align with previous studies, where two foliar applications (one at 35 days after planting and another at 50 days after planting) of *P. fluorescens* strain Pf3 (10^9 CFU/mL) effectively minimized jasmine leaf blight incidence (8). Similarly, an earlier study reported that *P. fluorescens*-1 was an efficient and cost-effective biocontrol agent for controlling *A. porri*, the pathogen responsible for purple blotch disease in onion (40). Additionally, antagonistic microorganisms like *Pseudomonas fluorescens* have demonstrated efficacy in inhibiting the growth of *Alternaria solani*, with soil applications significantly reducing disease incidence in pot culture experiments (41). In tomato plants, the liquid formulation of *Pseudomonas fluorescens* strain Pf1 significantly increased chitinase activity, which is crucial for breaking down the cell walls of *Fusarium oxysporum*, thereby restricting its invasion and reducing wilt incidence (42). Researchers evaluated the effectiveness of various oil cakes, including neem cake, pungam cake, groundnut cake, sesame cake and castor cake against *A. solani*, which causes fruit rot in the PKM1 tomato variety under pot culture conditions (43). Their findings revealed that applying neem cake at a rate of 10 g/kg of sterilized potting mixture improved yield (1.66 kg/plant) and plant growth (57.59 cm), while reducing disease incidence to 18.00 % over the control. Neem cake is rich in bioactive tetranorterpenoid lactones, including azadirachtin, nimbin, nimbidin, salanin and nimbolin B, with azadirachtin being the most potent as an antifeedant. Neem seed oil also contains

Table 5. Efficacy of different oil cakes on the mycelial growth of *A. jasmini* (I1)

S. No	Oil cakes	Concentrations (%)					
		10 %		15 %		20 %	
		Mycelial growth (mm)	Percentage reduction over	Mycelial growth (mm)	Percentage reduction over	Mycelial growth (mm)	Percentagereduction over control
1.	Castor	39.12^{f}	56.04	23.68^{e}	73.49	15.26^{e}	82.98
2.	Coconut	19.86^{c}	77.69	14.27^{c}	84.02	10.51^{c}	88.27
3.	Cotton	32.31^{e}	63.70	23.23^{e}	73.99	14.56^{e}	83.76
4.	Groundnut	25.27^{d}	71.61	19.51^{d}	78.16	12.70^{d}	85.84
5.	Mahua	22.23^{cd}	75.02	17.92^{d}	79.93	11.63^{cd}	87.03
6.	Neem	11.30^{a}	87.30	2.95^{a}	96.69	2.32^{a}	97.41
7.	Pungam	38.57^{f}	56.66	25.10^{e}	71.90	17.21^{f}	80.81
8.	Sesame	15.83^{b}	82.21	11.25^{b}	87.41	5.98^{b}	93.33
	Control	89.00^{g}	-	89.33^{f}	-	89.66^{g}	-
CD (P = 0.05 %)		**1.777**	-	**1.886**	-	**1.601**	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5 % level by DMRT.

Table 6. Evaluation of antagonists, plant oils and oil cake extracts against leaf blight of jasmine under pot culture condition

Treatment No.	Treatments	Disease incidence (%)	Percentage reduction over control
T_1	*P. indica* (AUPP23) (0.2 %)	26.74^{h} (31.13)	63.20
T_2	*B. subtilis* (Bs7) (0.2 %)	28.61^{i} (32.33)	60.63
T_3	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %)	24.32^{g} (29.54)	66.53
T_4	*P. indica* (AUPP23) (0.2 %) **+** Tulasi oil (1 %)	18.82^{d} (25.71)	74.10
T_5	*B. subtilis* (BS7) (0.2 %) + Tulasi oil (1 %)	20.16^{e} (26.67)	72.26
T_6	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + Tulasi oil (1 %)	15.64^{b} (23.29)	78.48
T_7	*P. indica* (AUPP23) (0.2 %) + Neem cake extract (10 %)	22.63^{f} (28.40)	68.86
T_8	*B. subtilis* (Bs7) (0.2 %) + Neem cake extract (10 %)	23.00^{f} (28.65)	68.35
T_9	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + Neem cake extract (10 %)	17.64^{c} (24.83)	75.73
T_{10}	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + Tulasi oil (1 %) + Neem cake extract (10 %)	13.55^{a} (21.59)	81.35
T_{11}	Mancozeb (0.2 %)	13.00^{a} (21.13)	82.11
T_{12}	Inoculated control	72.67^{j} (58.48)	-
	CD (P = 0.05 %)	**1.567**	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5 % level by DMRT.Values in the parentheses represent arc sine transformed values.

Fig. 8. Evaluation of antagonists, plant oils and oil cake extracts against leaf blight of jasmine under pot culture condition.

compounds such as nimbolides, olichinolide B and azadiradione, while neem leaves are a source of flavonoids like nimatone, quercetin, myricetin and kaempferol, along with azadirachtin, meliantrol, salanin, β-sitosterol and stigmasterol (39). Furthermore, GC-MS analysis conducted revealed that tulasi oil primarily comprises methyl eugenol (82.9 %), along with minor constituents such as β-caryophyllene (4.1 %), borneol (2.4 %), germacrene D (2.3 %) and α-copaene (1.9 %) (37). The predominant phenyl derivatives (83.8 %) were followed by sesquiterpene hydrocarbons (11.1 %), oxygenated monoterpenes (3.1 %), monoterpene hydrocarbons (0.6 %) and oxygenated sesquiterpenes (0.3 %). The presence of these bioactive compounds likely plays a significant role in inhibiting *A. jasmini*, reinforcing the findings of the present study.

Biological management of jasmine leaf blight under field condition

The findings on the effectiveness of antagonistic microorganisms, neem cake extract and plant oils in controlling jasmine leaf blight incidence are summarized in Table 7. The overall mean disease incidence for the treatments ranged from 07.44 to 78.67 %. Among the treatments T_6 (foliar application with *P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + tulasi oil (1 %) + neem cake extract (10 %)) recorded a minimum disease incidence of 09.97 % with maximum percentage reduction over control of 87.32 % and flower yield of 114.27 kg/15 cents which was on par with mancozeb (0.2 %), which recorded a PDI of 09.02 % with a maximum percentage reduction over control of 88.53 % and a yield of 115.56 kg/15 cents. This was followed by T_4, where foliar application of *P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + tulasi oil (1 %) resulted in a PDI of 10.45 % with an 86.71 % reduction in disease incidence over control, along with a yield of 112.56 kg/15 cents. Conversely, the highest disease incidence of 78.67 % was observed in T_8 (inoculated control), which yielded 72.47 kg/15 cents (Fig. 9). These findings align with those of a previous study which reported that foliar application of mancozeb (0.2 %), followed by *P. fluorescens* strains Pf3 (0.2 %) and Pf7 (0.2 %), effectively managed jasmine leaf blight (8). Several studies have reported that soil application of neem cake and vermicompost significantly reduced the severity of purple blotch in garlic by enhancing antagonistic microbial populations and inducing systemic resistance (44). Similarly, researchers noted that foliar application of *P. fluorescens* isolates significantly suppressed leaf spot disease in stevia caused by *A. alternate* (45). An earlier study reported that the untreated control plot exhibited the poorest growth parameters and the highest disease severity (46). In contrast, seedling treatment with *T. viride* + *P. fluorescens* + neem oil at 5 % resulted in the most favourable outcomes, demonstrating optimal growth parameters and the lowest disease intensity of *A. porri* (47).

Pseudomonas spp. mitigates pathogen-induced damage through direct mechanisms such as antibiosis and competition for iron, as well as by enhancing plant immunity via induced resistance (IR) (48). Additionally, it can be inferred that the production of various growth-promoting substances by

Table 7. Biological management of jasmine leaf blight under field condition

Treatment No.	Name of treatments	Disease incident (%)	Percentage reduction over control	Production of flower buds from 3rd- 45th days after 3rd spraying (kg/15 cents)*
T_1	*P. indica* (AUPP23) (0.2 %)	16.78^{e} (24.18)	78.67	86.89^{d}
T_2	*B. subtilis* (Bs7) (0.2 %)	18.91^{f} (25.77)	75.96	97.47^{c}
T_3	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %)	14.00^{d} (21.97)	82.20	100.11^{c}
T_4	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + Tulasi oil (1 %)	10.45^{b} (18.86)	86.71	112.56^{ab}
T_5	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + Neem cake extract (10 %)	12.33^{c} (20.55)	84.32	109.79^{b}
T_6	*P. indica* (AUPP23) (0.2 %) + *B. subtilis* (Bs7) (0.2 %) + Tulasi oil (1 %) + Neem cake extract (10 %)	09.97^{a} (18.40)	87.32	114.27^{a}
T_7	Mancozeb (0.2 %)	09.02^{a} (17.47)	88.53	115.56^{a}
T_8	Inoculated control	78.67^{g} (62.49)	-	72.47^{e}
	CD (P = 0.05 %)	**0.929**		**4.278**

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT. Values in the parentheses represent arc sine transformed values.

Fig. 9. Biological management of jasmine leaf blight under field condition

Pseudomonas spp., along with its antagonistic activity against *A. jasmini*, played a crucial role in promoting plant growth and increasing yield in this study (49). Researchers identified methyl eugenol (82.9 %) as the predominant compound in tulasi oil through GC-MS analysis, with β-caryophyllene (4.1 %), borneol (2.4 %), germacrene D (2.3 %) and α-copaene (1.9 %) as minor constituents (37). Neem contains several bioactive compounds, including azadirachtin, nimbin, nimbidin, salanin and nimbolin B, with azadirachtin being the most potent antifeedant.

The present study paves the better way for further testing of these two bio-agents, tulasi oil and neem cake, to find out the major metabolic compounds involved in hindering the pathogen growth which can be further used as efficient and better management practices against leaf blight pathogens.

Conclusion

In this study, the application of bioagents, plant oil and oil cake demonstrated a significant inhibitory effect against the fungal pathogen *Alternaria jasmini*. The results indicated that foliar application of *Pseudomonas indica* (AUPP23) at 0.2 %, *Bacillus subtilis* (Bs7) at 0.2 %, tulasi oil at 1 % and neem cake extract at 10 % on 60 and 75 days after planting provided the highest efficacy against jasmine leaf blight. Additionally, this treatment enhanced jasmine flower yield. These findings suggest that native biocontrol agents, plant oils and oil cakes have the potential to serve as effective tools for disease management, mitigating the harmful effects of phytopathogens on plant growth.

Authors' contributions

AM and AE conceived the idea and wrote the manuscript. TSR, PR, NR and MR supervised the work and contributed to the manuscript's design. CG and MSS carried out statistical analysis and generated the data. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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