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In vitro antifungal evaluation of various plant extracts against leaf blight disease of *Jasminum grandiflorum* caused by *Alternaria alternata* (Fr.) Keissler

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Abstract
Alternaria alternata is the wide spread pathogen causing leaf blight disease in Spanish jasmine (*Jasminum grandiflorum* L.) an important flower crop of commerce widely grown in India. Generous spectrum of effective fungicides is in use against this pathogen. The indiscriminate use of chemical fungicides is not only expensive but also hazardous for all living organisms. With this view the study was conducted to find a bio fungicide against *Alternaria alternata*. Twenty-four plant extracts and four oils were screened for antifungal activity under *in vitro* condition. Among the tested plant oils and plants products clove extract of *Allium sativum* (5%) recorded the highest reduction of the mycelial growth of 100% followed by leaf extract (10%) of *Datura metel* (68.44%) and oil (3%) of *Azadirachta indica* (59.88%). The effective plant extracts and oils have potential to be developed as potent fungicides in organic farming.

Keywords: *Jasminum grandiflorum* L., *Alternaria alternata*, Plant extracts and oils, Antifungal activity

Introduction
Jasmine is one of the leading traditional fragrant flower crop of Oleaceae family. Spanish jasmine (*Jasminum grandiflorum* L.) known as "Queen of the night" is the most fascinating, versatile flower crop of commerce and is a popular traditional loose flower (Mittal *et al.*,2011) [8]. It is extensively used for oil extraction and also for the preparation of jasmine concrete. India is the largest exporter of Jasmine oil in the world accounting for over 40 per cent of total world export (Arun *et al.*, 2016) [2]. In Tamil Nadu, *J. grandiflorum* is cultivated in area of 841ha with a production of 7569 tonnes and productivity of nine tonnes per hectare (tnhorticulture.tn.gov.in). It is affected by various plant pathogens of which the leaf blight disease reported to be one of the major diseases. First report on the incidence of leaf blight disease of *J. grandiflorum* caused by *Alternaria alternata* is given by Kamalalakshmi in 1996 [6]. Currently there is a indiscriminate use of synthetic fungicides for the management of plant diseases. The continuous use of fungicides is not only expensive but also hazardous for all living organisms. The alternate solution to reduce the use of chemicals in plant disease management is following the eco-friendly methods (Harish *et al*., 2008) [5]. Several higher plants and their products have shown success in controlling plant diseases and also proved to be harmless and non-toxic unlike chemical fungicides. Use of plant products in plant disease management assumes special significance by being an eco-friendly and cost-effective approach, this can also be used in integration with other management practices for a greater level of crop protection. (Talibi *et al.*, 2012) [16].

Materials and Methods
Collection and Isolation of the pathogen
A survey was conducted in *J. grandiflorum* growing areas of Tamil Nadu. The infected plant samples from the different locations were collected and brought to the laboratory for further studies. Leaves showing the characteristic target board symptoms were isolated by tissue segment method (Akhtar *et al.*, 2004) [1]. Infected leaf tissues with adjacent healthy portions were surface sterilized with 70 percent ethanol and washed with three changes of sterile distilled water. The surface sterilized bits were placed on PDA medium; the plates were maintained at 27 ± 1°C for seven days. Pure culture of the isolates maintained on PDA slants. (Ramjegathesh *et al.*, 2012) [10].

Pathogenicity test

Isolated pathogens were tested for the pathogenicity study. A month old *J. grandiflorum* plants were artificially inoculated with the conidial suspension of 2x106 cfu/ml and covered with a polythene bag for 24 hours to maintain the humidity. The plants inoculated with sterile distilled water served as control. After seven days of inoculation, these plants were observed for the disease symptom development and re-isolation of pathogen form the infected plants were done. Virulent isolate was taken for further studies. (Prathima *et al.*, 2018) [9]

Collection of Plant parts and oils

Plant parts were selected from the local flora on the basis of presence of antimicrobial properties according to literature or traditional knowledge, easy availability in bulk with very little commercial value. Leaves of karisalanganni, Tulasi, Periwinkle, Parthenium, Coleus, Dwarf copper leaf (Ponnangani), Eucalyptus, Lemon grass, veldt grape (Pirandai), Zimmu, Indian ginseng (Aswagandha), Indian mallow (Thuthi), Papaya, Neerium, Datura, Thoothuvalai, Prosopis, Henna, onion bulbs and garlic cloves were collected from AC&RI, Madurai. oils of common leucas (Thumbai) Mahua, Neem, Castor were collected from the local market.

Preperation of the plant extracts, bulbs, oils

The freshly collected plant products (Leaves, bulbs and cloves) were washed thoroughly with clean water and shade dried. About 20g of plant samples were ground using pestle and motor by adding 20 ml of sterlized distilled water. Extracts thus obtained were filtered using muslin cloth, then centrifuged with 10000× g for 15 min at 4°C. The supernatants were collected (Sallam *et al.,* 2012) [11]. The obtained supernatant formed 100 per cent extract and used for further dilutions.

Screening for the efficacy of plant extracts and oils against the *Alternaria alternata* by poison food technique

The requisite amount of the filtrate and oil was mixed in Potato Dextrose Agar (PDA) medium just before pouring to get the desired concentrations of leaf extracts (10%), Bulb extracts (5%), oils (3%) and gently shaken for thorough mixing of the extract into the PDA medium. The PDA plates containing plant extracts, bulbs, oils were inoculated with nine mm disc fresh culture of *Alternaria alternata.* PDA medium without any plant extracts, bulbs, oils served as control and incubated at 27 ± 1 °C temperature and three replicates were maintained for each treatment (Roshan *et al.,* 2014) [12]. Mycelial growth was measured in all the three treatments and compared with control. The percent inhibition of the fungus in treatments was calculated by following the below given formula.

$$\text{Percentage of inhibition} = \frac{A-B}{A} \times 100$$

Where,

A= Diameter of the pathogen in control, *B* = Diameter of the pathogen in treatment.

(Ravi Kumar *et al.,* 2013) [11]

Results and Discussion

Collection and Isolation of the pathogen

The data obtained during survey conducted in *J. grandiflorum* growing areas of Tamil Nadu are presented in table: 1. Leaves showing the typical symptoms of dark coloured lesion with concentric rings were collected and isolated (Fig: a). The isolated pathogen produced abundant brownish septate mycelium and conidia are olive- brown, produced in chains having both vertical and horizontal septations (fig: b, c, d, e). Based on the morphological characters, it is identified as *Alternaria* sp. and species level identification as *Alternaria alternata* was confirmed by molecular characterization by using the specific primer AaF (5'GTGCCTTCCCCCAAG GTCTCCG3') and AaR (5'CGGAAACGAGGTGGTTCAGGTC3'). The result obtained are in close association with Prathima *et al*., (2018) [7] who confirmed *Alternaria alternata* by using the same specific primer.

Pathogenicity test

The isolated pathogen was proved to be pathogenic on *J. grandiflorum* plants and identical disease symptoms were observed similar to the field symptoms. Leaves with severe coalescing of necrotic spots leads to drooping and withering of the entire plant. There were no symptoms on plants treated with sterile distilled water. Among the different isolates tested, isolate from the Andipatti village was the most virulent in inducing symptom and hence it was used for further studies. (Fig f,g).

Screening for efficacy of the plant extracts and oils against the *Alternaria alternata*

A total of 18 leaf extracts, onion bulb extract, garlic clove extract and four oils were evaluated against *Alternaria alternata* under *in vitro* (Fig.h). The data presented in the table (2) reveals that among different plant extracts and oils tested against the *Alternaria alternata*, *Allium sativum* clove extract (5%) recorded the highest reduction of the mycelial growth of 100% followed by leaf extracts (10%) of *Datura metel* (68.44%), *Abutilon indicum* (66.66%), *Alternanthera sessilis* (66.33%), *Prosopis juliflora* (65.55), *Allium tuberosum* (65.33) and *Lawsonia inermis* (63.11) The leaf extract of *Eucalyptus globulus* (27.77%) caused the lowest inhibition of the mycelial growth. Neem oil (3%) caused the reduction of 59.88% over control. The results obtained are supported by the findings of Lima *et al.,* (2016) [4] who have reported that garlic extract and the orange essential oil showed the potential to control *A.dauci* and *A.alternata***,** because their lower concentrations were able to sufficiently reduce the incidence of these fungi. The leaf extract of *D.metel, A.indica*, and *A.sativum* at 5% concentration caused the highest reduction of the mycelial growth of *A. solani* (Sallam *et al,* 2012) [13]. *In vitro* studies indicated that the leaf extract of Zimmu (*Allium cepa* L. *x Allium sativum* L.) demonstrated the highest inhibition of the mycelial growth (87%) of *A. solani*. (Satya *et al* 2005) [14]. Babu *et al*. (2000) [3] reported that, *Acacia concinna* pod extract recorded minimum per cent disease incidence (23.1%) followed by neem oil (30.9%) under field condition in tomato early blight caused by *Alternaria solani.* Similar results were reported by Taskeen *et al*. (2010) [15], Kumari et al (2006) [7], Ravikumar and Garampalli (2013) [11].

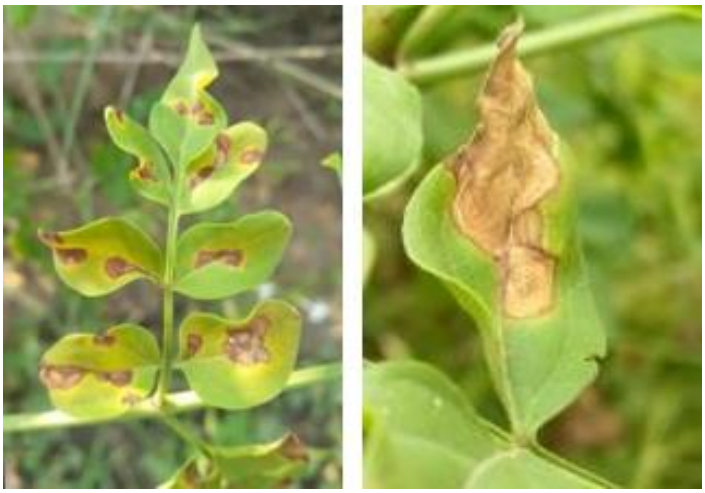

Fig a: *J. grandiflorum* leaf showing charecteristic Target board symptom

Table 1: Collection of leaf blight samples from *J.grandiflorum* plants from Tamil Nadu

S. No	Location	District	Isolate code	Latitude	Longitude
1	Andipatti	Dindugal	Ap	10.139	77.834
2	Milagaipatti	Dindugal	Mp	10.369	77.980
3	Othakadai	Madurai	Ok	9.939	78.121
4	Usilampatti	Madurai	Up	9.947	77.971
5	Poigaikulam	Madurai	Pg	10.055	78.194
6	Valayangulam	Madurai	Vk	9.818	78.092
7	Palanichettipatti	Theni	Pp	10.010	77.476
8	Thangachimadam	Ramanathapuram	Tm	9.369	78.830
9	Nagercoil	Kanyakumari	Nc	8.174	77.432
10	Palayankottai	Tirunelveli	Pk	8.741	77.694

Fig b: Culture of *Alternaria* sp. on PDA medium

Fig c: Mycelial growth of *Alternaria* sp.

Fig d: Microscopic view of conidia in chains

Fig e: Microscopic view of single conidium

Fig f: Pathogen uninoculated plant

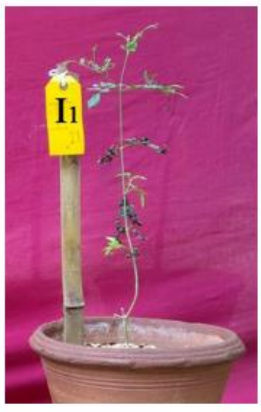

Fig g: Pathogen inoculated plant showing blighted leaves

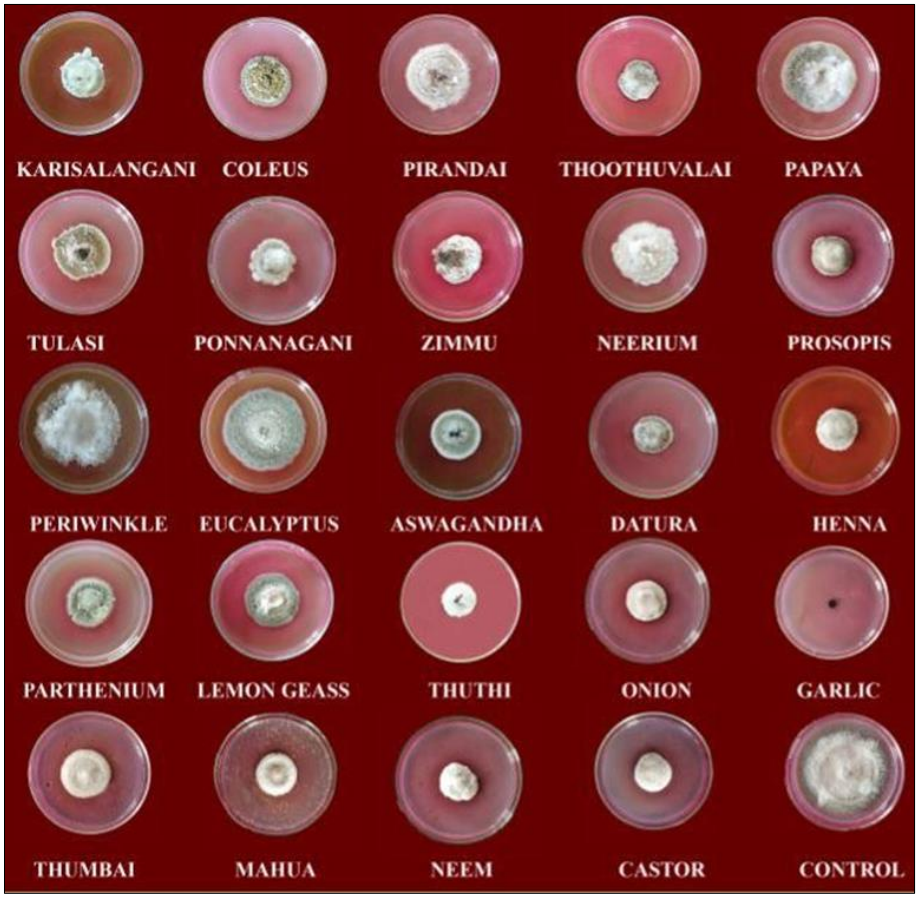

Fig h: *In vitro* efficacy of different plant extracts on mycelial growth of *Alternaria alternata*

Table 2: *In vitro* efficacy of different plant extracts on the mycelial growth of *Alternaria alternata*.

S. No	Plant name	Scientific name	Plant parts used	Extract (%)	Mycelial growth* (cm)	Percent inhibition over control
1.	Karisalanganni	*Eclipta prostrata*	Leaves	10	4.10gh	54.44
2.	Tulasi	*Ocimum sanctum*	Leaves	10	3.86ij	57.11
3.	Periwinkle	*Catharanthus roseus*	Leaves	10	6.64b	26.22
4.	*Parthenium*	*Parthenium hysterophorus*	Leaves	10	4.34f	51.77
5.	Coleus	*Coleus blumei*	Leaves	10	3.77jkl	58.11
6.	Ponnangani	*Alternanthera sessilis*	Leaves	10	3.03n	66.33
7.	Eucalyptus	*Eucalyptus globulus*	Leaves	10	6.50b	27.77
8.	Lemon grass	*Cymbopogon citratus*	Leaves	10	4.0hi	55.55
9.	Pirandai	*Cissus quadrangularis*	Leaves	10	5.30d	41.11
10.	Zimmu	*Allium cepa* × *Allium sativum*	Leaves	10	3.12n	65.33
11.	Aswagandha	*Withania somnifera*	Leaves	10	4.00hi	55.55
12.	Thuthi	*Abutilon indicum*	Leaves	10	3.01n	66.66
13.	Papaya	*Carica papayae*	Leaves	10	6.13c	31.88
14.	Neerium	*Nerium oleander*	Leaves	10	4.67e	48.11
15.	Datura	*Datura metel*	Leaves	10	2.84o	68.44
16.	Thoothuvalai	*Solanum trilobatum*	Leaves	10	3.57l	60.33
17.	Prosopis	*Prosopis juliflora*	Leaves	10	3.10n	65.55
18.	Henna	*Lawsonia inermis*	Leaves	10	3.32m	63.11
19.	Garlic	*Allium sativum*	Clove	5	0p	100
20.	Onion	*Allium cepa*	Bulb	5	3.32m	63.11
21.	Thumbai	*Leucas aspera*	Oil	3	4.29fg	52.55
22.	Mahua	*Madhuca longifolia*	Oil	3	4.27fg	52.33
23.	Neem	*Azadirachta indica,*	Oil	3	3.62kl	59.88
24.	Castor	*Ricinus communis*	Oil	3	3.89jk	56.78
25.	Control	Sterile water	Sterile water	-	9.00a	0

*Mean of three replication

Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

Conclusion

There are numerous reports available for the control of various phytopathogens and plant diseases under both *in vitro* and *in vivo* conditions by plant extracts. Results obtained from the current work showed that the plant extracts and oils screened against the *Alternaria alternata* exhibit antifungal activity. Plant extracts with antifungal activity are being explored in order to develop eco-friendly fungicide. In future use, on contrary to the problems associated with the use of fungicides, products derived from plants can be used as a new

alternative source to control the pathogens. Furthermore, studies are needed to determine the chemical nature of bioactive compounds present in the plant extracts that exhibited the antifungal activity.

References

1. Akhtar KP, Saleem MY, Asghar M, Haq MA. New report of *Alternaria alternata* causing leaf blight of tomato in Pakistan. Plant pathology. 2004; 53(6):816-816.
2. Arun M, Satish S, Anima P. Phyto pharmacological profile of *Jasminum grandiflorum* Linn. (Oleaceae). Chinese journal of integrative medicine. 2016; 22:311-20.
3. Babu S, Seetharaman K, Nanda Kumar R, Johnson I. Efficacy of fungal antagonists against leaf blight of tomato caused by *Alternaria solani*. Journal of Biological Control. 2000; 14:79-8.
4. Batista de Lima C, Lopes Assumpção Rentschler L, Tavares Bueno J, Cláudia Boaventura A. Plant extracts and essential oils on the control of *Alternaria alternata*, Alternaria dauci on the germination and emergence of carrot seeds (*Daucus carota* L.), 2016, 46.
5. Harish S, Saravanakumar D, Radjacommare R, Ebenezar EG, Seetharaman K. Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. Biocontrol. 2008; 53(3):555.
6. Kamalalakshmi C. Studies on leaf blight of Jathimalli (*Jasminum grandiflorum* L.) caused by *A. alternata* (Fr.) Keissler. M.Sc. Thesis. Tamil Nadu Agricultural University, Coimbatore, India, 1996.
7. Kumari L, Shekhawat KS, Rai PK. Efficacy of fungicides and plant extracts against Alternaria blight of periwinkle (*Catharanthus roseus*). Journal of Mycology and Plant Pathology. 2006; 36:134-137.
8. Mittal A, Sardana S, Pandey A. Ethnobotanical, phytochemical and pharmacological profile of *Jasminum sambac* (L.) Ait. Journal of Pharmaceutical and Biomedical Sciences. 2011; 11(11):1-7.
9. Prathima P, Thiruvudainambi S, Kalpana K. Molecular Characterization of *Alternaria alternata* (Fr.) Keissler Causing Leaf Blight Disease of Marigold. Int. J Pure App. Biosci. 2018; 6(6):1286-1291.
10. Ramjegathesh R, Ebenezar EG. Morphological and physiological characters of *Alternaria alternata* causing leaf blight disease of onion. International Journal of Plant Pathology. 2012; 3(2):34-44.
11. Ravikumar MC, Garampalli RH. Antifungal activity of plants extracts against *Alternaria solani*, the causal agent of early blight of tomato. Archives of phytopathology and plant protection. 2013; 46(16):1897-1903.
12. Roshan Regmi, Ravijha L, Sobita Simon, Abhilasha A, Lal. *In vitro* evaluation of some plant extracts against *Alternaria alternata* causing leaf spot of *aloe vera*. Arpn Journal of Agricultural and Biological Science. 2014; 9(10).
13. Sallam MA, Nas HWA, Kamal Abo-Elyousr AM. Evaluation of Various Plant Extracts against the Early Blight Disease of Tomato Plants under Greenhouse and Field Conditions. Plant Protect. Sci. 2012; 48(2):74-79.
14. Satya VK, Radha Jeyalakshmi R, Kavitha K, Paranidharan V, Bhaskaran R, Velazhahan R. *In vitro* antimicrobial activity of zimmu (*Allium sativum* L. *Allium cepa* L.) leaf extract. Archives of Phytopathology and Plant Protection. 2005; 38(3):185-192.
15. Taskeen UN, Wani AH, Mir RA. Antimycotic activity of plant extracts on the spore germination of some pathogenic fungi. Mycopath. 2010; 8(2):65-69.
16. Talibi I, Askarne L, Boubaker H, Boudyach EH, Msanda F, Saadi B *et al*. Antifungal activity of some Moroccan plants against *Geotrichum candidum*, the causal agent of post-harvest citrus sour rot. 2012; 35:41-46.