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Antagonistic activity of plant extracts against postharvest pathogens of major fruits from Dharwad, Karnataka region

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Abstract
Fruits are living entities and are highly perishable and one of the major reasons for postharvest losses is the spoilage due to postharvest fungal pathogens. In the present study, eight botanicals were tested *in vitro* against six postharvest pathogens *viz*., *Alternaria alternata*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Colletotrichum musae* and *Fusarium musae*. Inhibition of mycelial growth and spore germination was studied. Maximum mycelial growth inhibition of *A. alternata* (69.51%) by chromalaena leaf extract, *A. niger* (72.20%) by garlic bulb extract, *B. theobromae* (82.17%) by garlic bulb extract, *C. gloeosporioides* (68.91%) by garlic bulb extract, C. musae (84.80%) by neem leaf extract, *F. moniliforme* (62.20%) by tulasi leaf extract were recorded. Results of the study indicated that the for majority of the fungi, garlic bulb extract, neem leaf extract, tulasi leaf extract were effective in inhibition of mycelial growth and spore germination of the postharvest pathogens and can be incorporated into integrated management programmes.

Keywords: Postharvest pathogens - plant extracts - garlic - neem - tulasi

1. Introduction
Postharvest losses of perishable crops in developing countries have been estimated in the range of 5-50 per cent or more of the harvest (Salunke and Desai, 1984) [11]. Postharvest losses in mango (17-36%), banana (12-14%), citrus (8.30-30.70%), grapes (23-30%) have been reported from India (Madan and Ullasa, 1993) [6]. Postharvest diseases are traditionally controlled by chemicals but development of resistance in pathogens to fungicides and risk of fungicides towards public health and environment underlines the necessity to develop safe alternatives. There is an urgent need to develop novel and alternative postharvest disease management strategies. Non chemical management using plant extracts is opportunity for addressing the fungicide residue problems in the management of postharvest diseases. The present *in vitro* investigations were carried out in UAS, Dharwad to find out suitable plant extracts against various postharvest pathogens.

Materials and Methods
Fruits mango, banana, citrus, grapes and pomegranate showing symptoms of postharvest diseases) were collected from Dharwad market and nearby fields. Fungi were isolated by following standard tissue isolation method. Pathogenicity of the organisms was proved by proving Koch's postulates. Below mentioned botanical extracts were tested for their efficacy in inhibiting the mycelial growth and spore germination of various postharvest pathogens.

Antagonistic activity of the below mentioned plant extracts was tested *in vitro.*

Sl. No.	Scientific name	Vernacular name	Family	Part used
1	*Allium sativum* L.	Garlic	Amaryllidaceae	Bulb
2	*Azardirachta indica* Juss.	Neem	Meliaceae	Leaves
3	*Clerodendron inermii* Gaertn.	Kashmir bouquet	Verbenaceae	Leaves
4	*Chromolaena odoratum* L.	Communist weed	Compositae	Leaves
5	*Lantana camara* L.	Lantana	Verbenaceae	Leaves
6	*Ocimum sanctum* L.	Tulsi	Lamiaceae	Leaves
7	*Parthenium hysterophorous* L.	Congress grass	Compositae	Leaves
8	*Tridax procumbens* L.	Tridax	Compositae	Leaves and flowers

Preparation of stock solution of plant extracts
Fresh leaves/bulb of each plant extracts plant was collected and washed first in tap water and then in distilled water. Then, 100 g of fresh sample was crushed in a mixer grinder by adding

100 ml sterile distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Final filtrate thus obtained was used as stock solution.

i) Inhibition of mycelial growth

Antifungal activity of plant extracts at 10 per cent concentration in inhibiting the mycelial growth of postharvest fungal pathogens was tested using the poisoned food technique. Stock solutions of 10 ml was mixed with 90 ml of sterilized molten PDA medium respectively to get 10 per cent concentration. Twenty ml of the poisoned medium was poured into each of the 90 mm sterilized petriplates. Each plate was seeded with 0.5 cm mycelial discs taken from the periphery of eight day old fungal culture and Per cent inhibition of mycelial growth over control was calculated when the growth of the fungus is full in control plate by using the formula given by Vincent (1927) [16].

Inhibition of spore germination

Antifungal activity of plant extracts at 10 per cent concentration in inhibiting the spore germination of postharvest fungal pathogens was tested using the poisoned food technique. A single drop of the conidial suspension of the postharvest fungi was added to the wells of cleaned cavity slides, to which a single drop of different plant extracts of 20% concentration was added to get the required concentration of 10 per cent. The wells were immediately covered by using coverslips on the cavity slides and the periphery was smeared with vaseline. Control was maintained with distilled water. The cavity slides were kept in the petriplates lined with moist blotting paper and were incubated at room temperature. Observations were made from ten microscopic fields from each slide. Per cent germination was calculated from the number of total conidia and germinated conidia in each microscopic field. Further, the percent inhibition of spore germination was calculated by using the formula given by Vincent (1927) [16].

Results and Discussion

Antifungal activity of plant extracts was tested against six postharvest fungal pathogens *Alternaria alternata*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Colletotrichum musae* and *Fusarium moniliforme* was tested. The results on the effect of plant extracts on inhibition of mycelial growth and spore germination are presented below.

a. Inhibition of mycelial growth

Data pertaining to inhibition of mycelial growth of postharvest pathogens by plant extracts is presented in Table 1 & Fig 1. There was significant inhibition of mycelial growth of all the postharvest pathogens by all the plant extracts used in the study. Among the various plant extracts tested against *A. alternata*, maximum mycelial growth inhibition of 69.51 percent was observed in chromalaena leaf extract followed by neem leaf extract (55.49%), garlic bulb extract (54.01%). In case of *A. niger*, highest inhibition was observed in garlic bulb extract (72.20%) and neem leaf extract (71.10%), followed by tulasi leaf extract (64.40%). Similar results were obtained against *B. theobromae* with inhibitions of 82.17%, 56.45% and 52.14% with garlic bulb extract, neem leaf extract and tulsi leaf extract respectively. Maximum inhibition mycelial growth of *C. gloeosporioides* of 68.91% and 60.32% was recorded in garlic bulb extract and neem leaf extract respectively. Neem leaf extract (84.80%), garlic bulb extract (67.84%) and tulasi leaf extract (65.50%) were effective against *C. musae*. In case of *F. moniliforme*, tulasi leaf extract recorded highest inhibition of 62.20% followed by lantana leaf extract (52.98%) and neem leaf extract (52.58%).

b. Inhibition of spore germination

Data pertaining to inhibition of spore germination of postharvest pathogens by plant extracts is presented in Table 2 & Fig. 2. There was significant inhibition of spore germination of all the postharvest pathogens by all the eight plant extracts used in the study. Among the various plant extracts tested against *A. alternata*, maximum spore germination inhibition was observed in garlic bulb extract (70.89%) and tulasi leaf extract (69.97%), followed by neem leaf extract (55.89%). In case of *A. niger*, Highest inhibition of spore germination was observed in garlic bulb extract (78.09%) followed by tulasi leaf extract (71.80%) and neem leaf extract (71.10%). Spore germination of *B. theobromae* was inhibited maximum by neem leaf extract (81.10%), with garlic bulb extract (77.00%) followed by tulsi leaf (63.95%). Maximum inhibition of spore germination of *C. gloeosporioides* of 72.81% was recorded in garlic bulb extract followed by tulasi leaf extract (57.57%). Garlic bulb extract (74.00%) and tulasi leaf extract (73.35%) were effective against *C. musae*. In case of *F. moniliforme*, neem leaf extract recorded highest inhibition of 62.21% followed by tulasi leaf extract (55.91%).

Antifungal activity of various plant extracts against the pathogens under the present study were reported earlier by various workers. Antagonistic activity of garlic, (Jitender Singh and Majumdar, 2001; Karade and Sawant, 1999) [4, 5] neem and tusali (Prasanna Kumar, 2001; Jitender Singh and Majumdar, 2001) [6] against *A. alternata* was reported earlier. Antifungal activity against *A. niger* by neem and tulasi (Sobti *et al.*, 1995) [14], garlic (Yin *et al.*, 1998; Arun *et al*., 1995) [18, 2] was reported. Shirshikar (2002) [13] reported that 10% garlic bulb extract completely inhibited the mycelial growth and spore germination of *B. theobromae*. Antifungal activity of tulasi against *B. theobromae* was reported by Pathak (1997) [8] and Patil (1992) [9]. Effectiveness of garlic against *C, gloeosporioides* was reported by Shirshikar (2002) [13] where as that of neem and garlic was reported by Medha Chavan (1996) [7]. Oil extracted from tulasi has got antifungal properties against *C. musae* (Thoppil *et al.*, 2000) [15]. Antifungal activity of neem against *Fusarium spp*. was earlier reported by Dwivedi and Shukla (2000) [3] and Vir and Sharma (1985) [17].

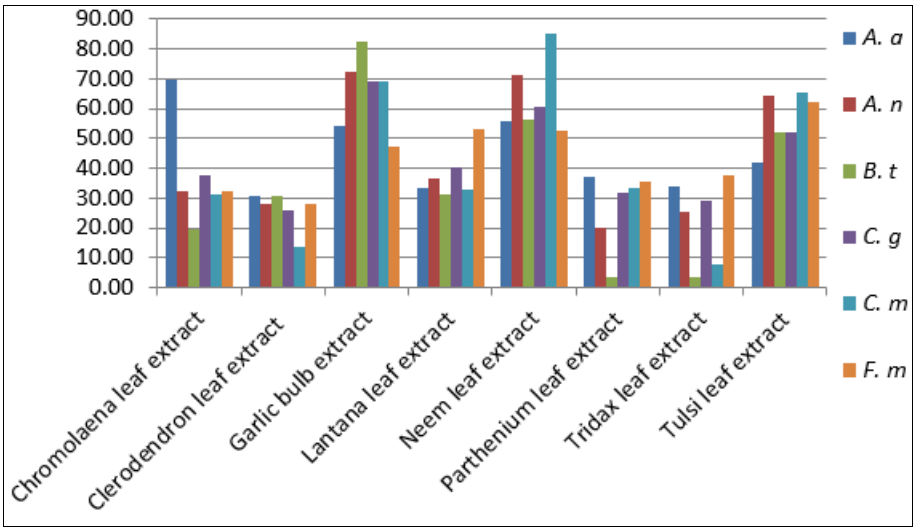
Antifungal activity of tulasi is reported to be due to thymol and phenol present in it, which are toxic to many pathogens (Anon., 1975) [1]. Patil (1992) [9] reported that tulsi contains polyamine biosynthesis inhibitor (s) which block the ornithine decarboxylase pathway in *B. theobromae* and *Rhizopus arrhizus*. Sharma and Prasad (1980) [12] reported that allicin (diallyl disulphide), allisatin I, allisatin II, garlicin, garlic phytoncide were the active principles that are involved in antifungal activity against many postharvest pathogens.

The present investigations also indicated the efficacy of garlic, neem and tulasi extracts against most of the postharvest pathogens. Hence, these can be used in integrated disease management strategies for control of postharvest diseases.

Table 1: Inhibition of mycelial growth of postharvest pathogens by plant extracts

S. No	Plant extract	Percent inhibition of mycelial growth					
		A. a	***A. n***	***B. t***	***C. g***	***C. m***	***F. m***
1	Chromolaena leaf extract	69.51 (56.50)	32.28 (34.56)	19.52 (4.53)	37.83 (37.99)	31.15 (33.88)	32.21 (34.56)
2	Clerodendron leaf extract	30.52 (33.51)	27.80 (31.81)	30.58 (5.62)	25.93 (30.59)	13.74 (21.74)	27.79 (31.70)
3	Garlic bulb extract	54.01 (47.31)	72.20 (58.19)	82.17 (9.12)	68.91 (56.11)	68.87 (55.48)	47.22 (43.72)
4	Lantana leaf extract	33.12 (35.11)	36.32 (37.04)	31.37 (5.69)	40.32 (39.44)	32.70 (34.99)	52.98 (46.70)
5	Neem leaf extract	55.49 (48.17)	71.12 (57.49)	56.45 (7.58)	60.32 (50.96)	84.80 (67.08)	52.58 (46.66)
6	Parthenium leaf extract	37.00 (37.47)	20.00 (26.56)	3.24 (2.06)	31.93 (34.03)	33.40 (35.25)	35.32 (36.36)
7	Tridax leaf extract	33.71 (35.46)	25.22 (30.12)	3.24 (2.06)	29.32 (32.74)	7.82 (16.19)	37.60 (37.78)
8	Tulsi leaf extract	42.12 (40.46)	64.40 (53.31)	52.14 (7.29)	52.23 (46.26)	65.50 (54.07)	62.20 (52.06)
9	Mean	44.36 (41.75)	43.29 (41.13)	29.25 (5.50)	43.09 (41.01)	41.00 (39.83)	43.40 (41.20)
10	CD at 1% Level	1.45	2.08	1.26	2.74	2.31	2.50
11	S.Em ±	0.37	0.53	0.07	0.70	0.59	0.64

A. a - Alternaria alternata, A. n - Aspergillus niger, B. t - Botryodiplodia theobromae,
C. g - Colletotrichum gloeosporioides, C. m - Colletotrichum musae, F. m - Fusarium moniliforme
*Figures in the parentheses are angular transformed values



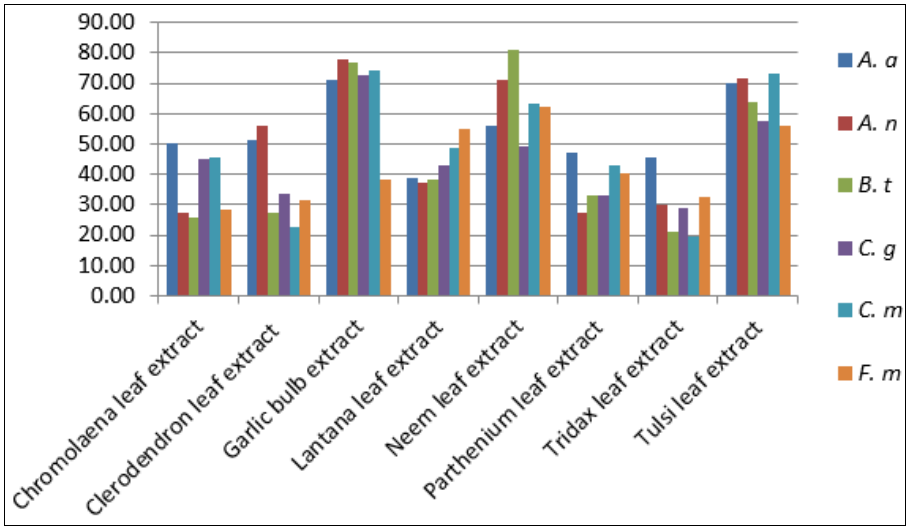
A. a - Alternaria alternata, A. n - Aspergillus niger,
B. t - Botryodiplodia theobromae, C. g - Colletotrichum gloeosporioides, C. m - Colletotrichum musae, F. m - Fusarium moniliforme

Fig 1: Percent inhibition of mycelial growth of postharvest pathogens by plant extracts

Table 2: Inhibition of spore germination of postharvest pathogens by plant extracts

S. No	Plant extract	Percent inhibition of spore germination					
		A. a	***A. n***	***B. t***	***C. g***	***C. m***	***F. m***
1	Chromolaena leaf extract	50.23 (45.13)	27.40 (31.56)	26.00 (30.68)	45.26 (42.21)	45.44 (42.39)	28.42 (32.22)
2	Clerodendron leaf extract	51.12 (45.58)	56.12 (48.47)	27.13 (31.33)	33.45 (35.25)	22.57 (28.34)	31.60 (34.31)
3	Garlic bulb extract	70.89 (57.33)	78.09 (62.02)	77.00 (61.30)	72.81 (58.57)	74.00 (59.12)	38.09 (38.57)
4	Lantana leaf extract	38.69 (38.48)	37.34 (37.69)	38.12 (38.10)	43.10 (40.96)	48.54 (44.10)	55.15 (47.90)
5	Neem leaf extract	55.89 (48.37)	71.10 (56.41)	81.10 (64.24)	49.39 (44.62)	63.21 (52.85)	62.21 (52.07)
6	Parthenium leaf extract	47.15 (43.35)	27.10 (31.34)	32.82 (34.98)	33.12 (35.11)	43.01 (40.96)	40.24 (39.09)
7	Tridax leaf extract	45.67 (42.45)	29.67 (32.92)	21.13 (27.34)	28.69 (32.35)	19.39 (26.04)	32.30 (34.63)
8	Tulsi leaf extract	69.97 (56.75)	71.80 (57.92)	63.95 (53.10)	57.57 (49.34)	73.35 (58.98)	55.91 (48.40)
9	Mean	53.80 (47.18)	49.82 (44.91)	45.90 (42.63)	45.00 (42.30)	48.00 (44.09)	42.90 (40.93)
10	CD at 1% Level	2.03	2.13	2.42	1.87	1.86	2.14
11	S.Em ±	0.52	0.54	0.62	0.48	0.48	0.55

A. a - Alternaria alternata, A. n - Aspergillus niger, B. t - Botryodiplodia theobromae,
C. g - Colletotrichum gloeosporioides, C. m - Colletotrichum musae, F. m - Fusarium moniliforme
*Figures in the parentheses are angular transformed values



A. a - Alternaria alternata, A. n - Aspergillus niger,
B. t - Botryodiplodia theobromae, C. g - Colletotrichum gloeosporioides, C. m - Colletotrichum musae, F. m - Fusarium moniliforme

Fig 2: Percent inhibition of spore germination of postharvest pathogens by plant extracts

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