Research Article

doi: 10.25081/jpc.2018.v46.i2.3720
Journal of Plantation Crops, 2018, 46(2): 92-101

Evaluation of plant extracts for antifungal activity against *Colletotrichum gloeosporioides*, the incitant of leaf blight in small cardamom and anthracnose of black pepper

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(Manuscript Received: 15-12-17, Revised: 03-07-18, Accepted: 12-07-18)

Abstract

Stress imposed by biological entities is considered as the major production constraint encountered by black pepper and small cardamom in India and elsewhere. Among the fungal diseases, leaf blight and anthracnose incited by *Colletotrichum gloeosporioides* in cardamom and black pepper, respectively, are the most prevalent and economically important diseases. In the present study, 35 plant species were evaluated to assess antifungal property against the targeted pathogen under *in vitro* conditions. Phytoextracts of *Solanum nigrum* (5%), *S. torvum* (20%) and *Azadirachta indica* (5%) exhibited maximum inhibitory effect whereas, *Leucas aspera*, *Costus igneus*, *Datura stramonium*, *Lantana camara*, *Glycosmis pentaphylla* and *Adhatoda vasica* promoted growth of the pathogen. Microscopic observations revealed abnormal morphological and structural alterations of hyphae, including increase in size and number of vacuoles, anomalous branching and abnormal swelling at hyphal tips. Information emanated from the present study indicates that, the efficacious plant species identified as potential sources of bioactive antifungal molecules could be further exploited to devise management strategies based on bio-prospecting.

Keywords: Antifungal activity, black pepper, *Colletotrichum gloeosporioides*, hyphal malformation, plant extract, small cardamom

Introduction

Black pepper (*Piper nigrum* L.) and small cardamom (*Elettaria cardamomum* Maton), the "King" and "Queen" of spices respectively, occupy unique positions in the global spice industry, owing to their inherent superior qualities and multifarious uses. These spices have been indispensible components in various industries such as food, confectionary, medicinal, perfumery, cosmetics and contributes substantially to the foreign exchequer (Nybe *et al*., 2007). Besides its centre of origin, India, these crops have been widely adopted and cultivated on a commercial scale in other South East Asian countries such as Vietnam, Indonesia, Guatemala, Malaysia and Sri Lanka. Globally, Guatemala occupies the topmost rung in cardamom production, while Vietnam is the leader in black pepper production (Ravindran, 2000; 2002). In India, cardamom and black pepper cultivation is mainly confined to the southern agro-ecological zones *viz*., Karnataka, Kerala and Tamil Nadu.

Leaf blight and anthracnose/spike shedding incited by *Colletotrichum* spp. in cardamom (Chethana *et al*., 2016) and black pepper (Chethana *et al*., 2015) respectively, is reported to be a pernicious problem, occurring widespread across geographical regions and have been a major cause of concern to the industry (Thomas and Bhai, 2002; Praveena *et al*., 2013; Biju *et al*., 2013). Perennial nature of the crops, large-scale cultivation of susceptible varieties and pre-disposing factors such as conducive weather pattern and micro-climatic conditions coinciding with vulnerable stages of the crop provides a favourable niche for the establishment and proliferation of *C. gloeosporioides* in cardamom and black pepper-based cropping systems.

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Though integrated disease management (IDM) packages have been evolved and recommended to ward-off these diseases, application of synthetic fungicides has been considered as the most effective as well as preferred strategy. However, besides the high input costs, indiscriminate and non-judicious application of synthetic molecules may lead to accumulation of residues in the environment, resulting in potential health hazards and also might result in the evolution of resistant strains in field populations of the pathogen. A broad spectrum of indigenous plant species have been reported to be the reservoirs of innocuous biologically active molecules with potent antimicrobial properties and could be used as an alternative to the currently recommended synthetic chemicals. Formulating an IDM module by incorporating locally available plant genera to manage plant diseases is highly imperative to protect the environment, prevent the evolution of new strains of pathogens and moreover, to manage the diseases effectively and eco-friendly (Gurjar *et al*., 2012). Inhibition of *C. gloeosporioides* and related species with plant extracts under *in vitro* and field conditions in diverse plant species of economic importance are reported (Tasiwal *et al*., 2009; Masangwa *et al*., 2013; Alemu *et al*., 2014). However, pertinent information on the evaluation of plant species for their antimicrobial properties against *C. gloeosporioides* infecting cardamom and black pepper and the possible mode of action are inadequate. Hence, the present investigation was formulated with the objectives (1) to evaluate different locally available plant species against *C. gloeosporioides* for antifungal efficacy under laboratory conditions and (2) to study the mechanism of inhibitory action induced by the most efficacious plant species.

Materials and methods

The fungal isolates used in the present study were collected from the experimental farm, ICAR-Indian Institute of Spices Research (IISR) Regional Station, Appangala, Kodagu, Karnataka. The pathogen, *Colletotrichum gloeosporioides* was isolated from blight affected leaves of cardamom and black pepper foliage, exhibiting characteristic anthracnose symptoms. The pathogens were isolated from symptomatic leaves by adopting standard isolation procedures on potato dextrose agar (PDA) medium supplemented with streptomycin sulphate to prevent contamination due to bacteria. The plates were subsequently incubated at 25±1 °C under continuous illumination. Hyphal strands originating from the leaf bits were aseptically transferred to PDA slants (amended with streptomycin sulphate) and maintained at 4 °C for further studies. The pathogen isolated from infected samples was identified based on colony and conidial characters.

The plant species were selected on the basis of easy availability throughout the year in the field, which were apparently free from diseases indicated by absence of symptoms on different plant parts. Crude extracts were prepared from both young and older leaves as well as from the tender shoots of 35 plant species listed in Table 1. Freshly harvested leaves and tender shoots were thoroughly washed with tap water initially followed by sterile distilled water, air-dried at 27±1 °C and 100 g of cleaned plant parts were homogenized with 100 mL sterile distilled water in a homogenizer. The resultant homogenate was filtered through double-layered muslin cloth and the resultant filtrate was subsequently centrifuged at 1300 g for 15 minutes. The supernatant thus obtained was used as the stock solution for bioassay experiments (Ogbebor *et al*., 2007; Shovan *et al*., 2008).

The antifungal property of plant species was assessed by employing poisoned food technique. 2.5, 5, 10 and 20 mL of the stock solution were mixed with 97.5, 95, 90 and 80 mL of sterilized molten PDA in 250 mL conical flasks respectively, so as to get 2.5, 5, 10 and 20 per cent concentrations and thoroughly shaken to get a uniform mixture. A 20 mL poisoned medium thus prepared was poured into each of the 90 mm Petridishes. Each plate was subsequently seeded with mycelial discs of 5 mm diameter derived from the advanced border of actively growing five days old culture of the pathogen. The discs were placed at the center of each Petridish and each treatment was replicated thrice. The plates were incubated at 25±1 °C and observations on radial growth of the colony were recorded when maximum growth was occurred in the control plates.

The efficacy of plant extracts was assessed based on per cent inhibition of the colony growth, calculated using the formula:

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

where, I = per cent inhibition, C = radial growth in control and T = radial growth in treatment.

To study the effect of phyto-extracts on hyphal growth and morphological alterations, the hyphal strands were carefully picked from the advancing margin of the colony from the treatment as well as control plates. The hyphal strands from culture plates were mounted in water and microscopic examinations were carried out using fresh direct mounts after staining with lactophenol cotton blue. The slides were observed for morphological alterations at 10X and 40X magnifications and photographed.

The *in vitro* bioassay experiments were laid out in completely randomized design (CRD) and the data recorded in per cent values were subjected to arcsine transformation. The transformed data were statistically analyzed using the software package AGRES version 7.01 @ 1994 Pascal Intl Software Solutions.

Results and discussion

The results of present investigation indicated that, different plant species evaluated exhibited varied antifungal potentials. Though complete inhibition of *C. gloeosporioides* was not observed with any of the plant extracts tested, significant level of inhibition could be noticed with few species. Extracts of four

Table 1. List of plant species used for *in vitro* assay for antifungal efficacy

Common name	Scientific name	Family
Wild sage	*Lantana camara*	Verbenaceae
Common Leucas/ Thumba	*Leucas aspera*	Lamiaceae
Indian wormwood/ Mugwort	*Artemisia nilagirica*	Asteraceae
Ban nimbu/Paanal	*Glycosmis pentaphylla*	Rutaceae
Gliricidia	*Gliricidia maculata*	Fabaceae
Holy basil/Tulasi	*Ocimum sanctum*	Lamiaceae
Herb of grace/common rue	*Ruta graveolens*	Rutaceae
Hairy spurge	*Euphorbia hirta*	Euphorbiaceae
Prickly nightshade	*Solanum torvum*	Solanaceae
Barbados nut/Purging nut	*Jatropha curcas*	Euphorbiaceae
Castor	*Ricinus communis* (green)	Euphorbiaceae
Castor	*Ricinus communis* (pink)	Euphorbiaceae
Spiral flag/Insulin plant	*Costus igneus*	Costaceae
Chaste tree	*Vitex negundo*	Verbenaceae
Malabar nut	*Adhatoda vasica*	Acanthaceae
Bitter bush	*Chromolaena odorata*	Asteraceae
Amaranthus	*Amaranthus* spp.	Amaranthaceae
Henna	*Lawsonia inermis*	Asteraceae
Mad apple/Devils weed	*Datura stramonium*	Solanaceae
Water willow/Shrimp plant	*Justicia wyanaadensis*	Acanthaceae
Four O' clock plant	*Mirabilis jalapa* (pink)	Amaranthaceae
Garden nightshade	*Solanum nigrum*	Solanaceae
Sweet Marjoram	*Coleus aromaticus*	Lamiaceae
Sweet broomweed	*Scoparia dulcis*	Scrophulariaceae
Neem	*Azadirachta indica*	Meliaceae
Sickle senna/Tora	*Cassia tora*	Caesalpinaceae
Chinese Wedelia	*Wedelia chinensis*	Asteraceae
Periwinkle	*Catharanthus roseus* (pink)	Apocyanaceae
Periwinkle	*Catharanthus roseus* (white)	Apocyanaceae
Four o' clock plant	*Mirabilis jalapa* (white)	Nyctagenaceae
Pudina	*Mentha arvensis*	Lamiaceae
Bottle brush	*Callistemon lanceolatus*	Myrtaceae
Yellow oliander	*Thevetia peruviana*	Apocyanaceae
Large cardamom	*Amomum subulatum*	Zingiberaceae
Yellow berried night shade	*Solanum xanthocarpum*	Solanaceae

plants *viz*., *Solanum nigrum*, *S. torvum*, *Mirabilis jalappa* and *Azadirachta indica* showed more than 50 per cent inhibition of mycelial growth of *C. gloeosporioides*. Of the 35 plant species screened, extracts of *Costus*, *Datura*, *Leucas*, *Glycosmis* and *Lantana* did not exhibit any inhibition on mycelial growth of the pathogen. The ineffective plant genera, even at the higher doses tested, behaved as nutrient substrates promoting mycelial growth of the pathogen. The growth promotional activity may be attributed to the presence of some vital constituents essential for growth of *C. gloeosporioides*. The variation in inhibitory effect observed among the plant species could be explained by a substantial difference in the concentration of various bio-active molecules (Bonzi *et al*., 2012). Ineffectiveness of *O. sanctum* leaf extract against *C. gloeosporioides* is supported by earlier report of Patel and Joshi (2001).

The pathogen isolated from anthracnose affected black pepper and cardamom leaves with leaf blight symptoms was identified as *Colletotrichum gloeosporioides* based on colony and conidial characters (Fig. 1a and 1 b). The extracts of 35 plant species when tested against cardamom leaf blight pathogen showed mycelial inhibition under *in vitro* condition (Table 2). Among all the plant species evaluated, extract of *S. torvum* was found to be highly inhibitory to *C. gloeosporioides* (Fig. 2). The extract at 5, 10 and 20 per cent concentrations resulted in 58.9, 61.5 and 70.4 per cent inhibition of radial growth, respectively. However, the extract of *S. nigrum* was found less effective compared with *S. torvum.* The lower concentrations (2.5% and 5%) of *S. nigrum* was found to be inhibitory than the higher concentrations. *S. nigrum* at 5 per cent concentration showed maximum inhibition of mycelial growth (60%) (Fig.3). *A. indica* at lower concentrations of 2.5 and 5 per cent showed inhibition of 50.7 and 58.5 per cent, respectively than at higher concentrations (Fig. 4). Extract of *M. jalappa* at higher concentrations (10 and 20%) was found to impede

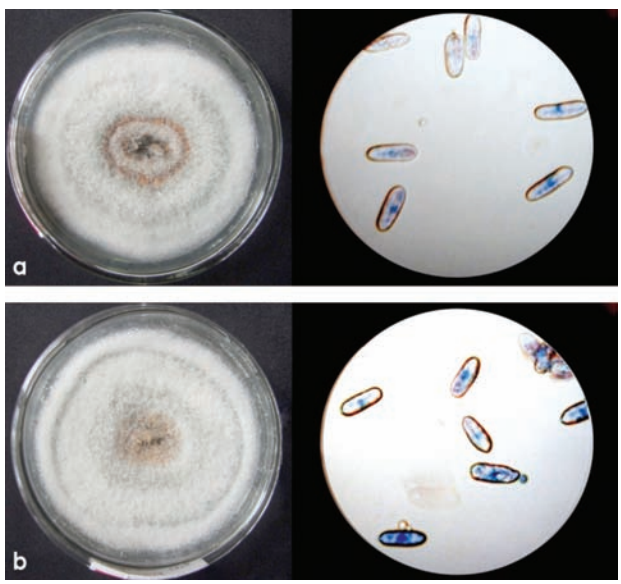

Fig. 1. Colony and conidial characters of *Colletotrichum gloeosporioides* infecting (a) black pepper and (b) cardamom

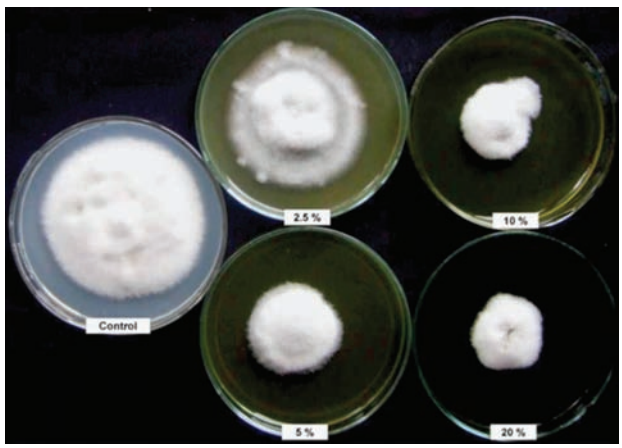

Fig. 2. *In vitro* antifungal activity of *Solanum torvum* extract on cardamom isolate of *Colletotrichum gloeosporioides*

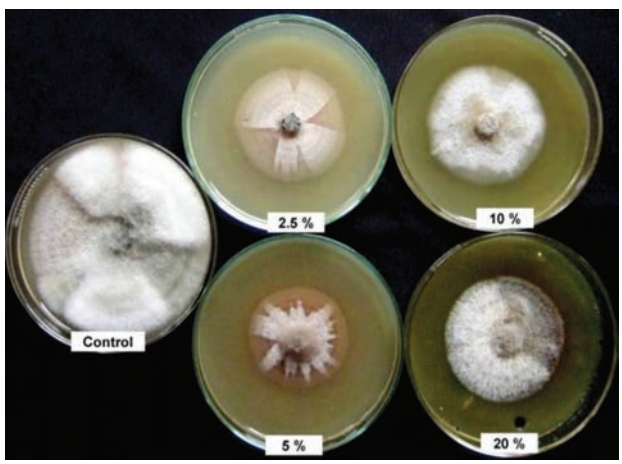

Fig. 3. *In vitro* antifungal activity of *Solanum nigrum* extract on cardamom isolate of *Colletotrichum gloeosporioides*

Table 2. Per cent mycelial growth inhibition of *Colletotrichum gloeosporioides* infecting small cardamom by various plant extracts

Plant species	Concentration of plant extract (%)			
	2.5	5	10	20
Lantana camara	0 (0) *	7.7 (16.2)	10.7 (19.1)	15.6 (23.2)
Leucas aspera	0 (0)	1.8 (7.8)	27.4 (31.6)	32.2 (34.6)
Artemisia nilagirica	3.7 (11.1)	11.8 (20.1)	15.2 (22.9)	27.8 (31.8)
Glycosmis pentaphylla	3.7 (11.1)	3.7 (11.1)	11.9 (20.1)	14.8 (22.6)
Ocimum sanctum	12.9 (21.1)	7.4 (15.8)	9.6 (18.1)	23.3 (28.9)
Ruta graveolens	27.1 (31.4)	13.7 (21.7)	20.0 (26.6)	23.7 (29.1)
Glyricidia maculata	4.8 (12.7)	6.7 (14.9)	10.4 (18.8)	18.5 (25.5)
Solanum torvum	31.1 (33.9)	58.9 (50.1)	61.5 (51.7)	70.4 (57.1)
Jatropha curcus	16.3 (23.8)	14.8 (22.6)	20.4 (26.8)	29.6 (32.9)
Ricinus communis (green)	12.6 (20.8)	19.6 (26.3)	23.7 (29.1)	20.4 (26.8)
Ricinus communis (purple)	25.6 (30.4)	21.1 (27.3)	23.3 (28.9)	26.7 (31.1)
Euphorbia hirta	9.3 (17.7)	17.4 (24.7)	16.7 (24.1)	32.6 (34.8)
Costus igneus	0 (0)	0 (0)	4.4 (12.2)	8.9 (17.4)
Vitex negundo	2.6 (9.3)	6.3 (14.5)	13.3 (21.4)	21.5 (27.6)
Adhatoda vasica	11.1 (19.5)	10.0 (18.4)	2.6 (9.3)	6.7 (14.9)
Chromolena odorata	1.5 (6.9)	14.4 (22.3)	11.5 (19.8)	24.1 (29.4)
Lawsonia inermis	16.7 (24.1)	17.4 (24.7)	20.0 (26.6)	32.2 (34.6)
Datura stramonium	2.2 (8.6)	5.6 (13.6)	7.0 (15.4)	23.7 (29.1)
Justicia wayanadensis	2.6 (9.3)	7.7 (16.2)	39.3 (38.8)	40.4 (39.5)
Mirabilis jalapa (pink)	37.8 (37.9)	22.2 (28.1)	44.1 (41.6)	59.3 (50.4)
Solanum nigrum	49.6 (44.8)	60.0 (50.8)	44.1 (41.6)	42.9 (40.9)
Coleus aromaticus	14.8 (22.6)	29.6 (32.9)	40.7 (39.7)	37.8 (37.9)
Scoparia dulcis	9.3 (17.7)	9.6 (18.1)	14.4 (22.3)	22.2 (28.1)
Azadirachta indica	50.7 (45.4)	58.5 (49.9)	23.7 (29.1)	39.6 (39.0)
Solanum xanthocarpum	22.2 (28.1)	16.6 (24.1)	20.0 (26.6)	6.7 (14.9)
Wadelia chinensis	28.5 (32.3)	25.56 (30.38)	37.8 (37.9)	31.1 (33.9)
Catharanthus roseus (pink)	11.1 (19.5)	16.30 (23.82)	17.4 (24.67)	29.6 (32.9)
Catharanthus roseus (white)	46.7 (43.1)	23.70 (29.14)	22.2 (28.1)	14.8 (22.6)
Mirabilis jalapa (white)	17.4 (24.7)	17.04 (24.39)	21.9 (27.9)	16.7 (24.1)
Thevetia peruviana	14.1 (22.1)	21.85 (27.88)	33.3 (35.3)	33.3 (35.3)
Mentha arvensis	12.9 (21.1)	11.85 (20.14)	21.1 (27.4)	23.7 (29.1)
Amaranthus spp.	20.0 (26.6)	17.41 (24.67)	20.0 (26.6)	28.9 (32.5)
Cassia tora	31.1 (33.9)	34.81 (36.17)	29.6 (32.99)	33.7 (35.5)
Callistemon lanceolatus	28.1 (32.1)	28.15 (32.06)	34.1 (35.7)	35.9 (36.8)
Amomum subulatum	28.9 (32.5)	32.59 (34.83)	32.6 (34.8)	16.7 (24.1)
	S. Ed	**CD (0.05%)**	**CD (0.01%)**	
Plant extract (P)	**1.5**	**2.9**	**3.9**	
Concentration (C)	**0.5**	**1.0**	**1.3**	
P X C	**3.0**	**5.9**	**7.8**	

*Figure in the parentheses represents arcsine transformed values

growth of *C. gloeosporioides* (44.1% and 59.3%) whereas, *Catharanthus roseus* at 2.5 per cent concentration showed 46.7 per cent inhibition. Out of the 35 plant species evaluated, 14 showed less than 25 per cent inhibition of mycelial growth of the pathogen at all concentrations tested. Extracts of *Lantana*, *Leucas* and *Costus* at lower concentrations did not adversely affect growth kinetics of the pathogen.

The results of present study demonstrated that, mycelial growth of *C. gloeosporioides* infecting black pepper was significantly inhibited by extracts of *S. nigrum*, *S. torvum* and *A. indica* at different concentrations (Table 3). Extract of *S. nigrum* at all concentrations tested was found inhibitory, which ranged from 59.63-74.80 per cent. The extract resulted in more than 70 per cent inhibition at concentrations of 5, 10 and 20 per cent. Maximum

Table 3. Per cent mycelial growth inhibition of *Colletotrichum gloeosporioides* infecting black pepper by various plant extracts

Plant species	Concentration of plant extract (%)			
	2.5	5	10	20
Lantana camara	4.4 (12.1)*	7.0 (15.4)	8.5 (16.9)	3.7 (11.1)
Leucas aspera	0 (0)	2.2 (8.6)	3.3 (10.5)	44.1 (41.6)
Artemisia nilagirica	11.1 (19.5)	7.4 (15.8)	5.6 (13.6)	9.6 (18.1)
Glycosmis pentaphylla	13.7 (21.7)	1.9 (7.8)	4.4 (12.2)	19.6 (26.3)
Ocimum sanctum	7.4 (15.8)	7.4 (15.8)	28.5 (32.3)	24. (29.6)
Ruta graveolens	6.7 (14.9)	27.8 (31.8)	24.8 (29.9)	29.6 (32.9)
Glyricidia maculata	7.4 (15.8)	8.9 (17.4)	8.9 (17.4)	14.4 (22.3)
Solanum torvum	31.5 (34.1)	48.2 (43.9)	53.7 (47.1)	65.6 (54.1)
Jatropha curcus	5.6 (13.6)	8.5 (16.9)	11.1 (19.5)	11.1 (19.5)
Ricinus communis (green)	15.9 (23.5)	13.7 (21.7)	10.4 (18.8)	11.9 (20.1)
Ricinus communis (purple)	10.4 (18.8)	9.3 (17.7)	8.9 (17.4)	8.2 (16.6)
Euphorbia hirta	17.0 (24.4)	14.8 (22.6)	7.0 (15.4)	5.6 (13.6)
Costus igneus	0 (0)	0.7 (4.9)	10.0 (18.4)	12.2 (20.5)
Vitex negundo	5.6 (13.6)	3.3 (10.5)	1.5 (6.9)	9.3 (17.7)
Adhatoda vasica	14.8 (22.6)	10.4 (18.8)	7.8 (16.2)	8.5 (16.9)
Chromolena odorata	2.9 (9.9)	11.8 (20.1)	14.1 (22.0)	18.2 (25.2)
Lawsonia inermis	10.0 (18.4)	16.3 (23.8)	21.5 (27.6)	37.0 (37.5)
Datura stramonium	0 (0)	0 (0)	0 (0)	6.7 (14.9)
Justicia wayanadensis	4.4 (12.2)	0.4 (3.5)	36.7 (37.3)	41.1 (39.9)
Mirabilis jalapa (pink)	29.6 (32.9)	24.1 (29.4)	36.3 (37.1)	58.5 (49.9)
Solanum nigrum	59.6 (50.6)	74.8 (59.9)	72.2 (58.2)	71.1 (57.5)
Coleus aromaticus	27.8 (31.8)	22.9 (28.6)	39.6 (39.0)	38.9 (38.6)
Scopariadulcis	14.8 (22.7)	14.8 (22.6)	20.4 (26.8)	28.9 (32.5)
Azadirachta indica	57.8 (49.5)	59.6 (50.6)	39.6 (39.0)	51.1 (45.7)
Solanum xanthocarpum	9.3 (17.7)	5.6 (13.6)	5.6 (13.6)	31.1 (33.9)
Wadelia chinensis	17.1 (24.4)	31.1 (33.9)	25.6 (30.4)	33.3 (35.3)
Catharanthus roseus (pink)	18.9 (25.8)	21.5 (27.6)	24.1 (29.4)	26.3 (30.9)
Catharanthus roseus (white)	45.6 (42.5)	31.1 (33.9)	28.5 (32.3)	21.1 (27.4)
Mirabilis jalapa (white)	22.6 (28.4)	19.3 (26.0)	22.2 (28.1)	26.3 (30.9)
Thevetia peruviana	28.5 (32.3)	15.6 (23.2)	17.0 (24.4)	16.7 (24.1)
Mentha arvensis	12.6 (20.8)	10.7 (19.1)	19.3 (26.0)	21.9 (27.9)
Amaranthus spp.	30.4 (33.5)	25.9 (30.6)	20.4 (26.8)	20.0 (26.6)
Cassia tora	30.4 (33.5)	31.1 (33.9)	31.5 (34.1)	28.5 (32.3)
Callistemon lanceolatus	27.8 (31.8)	31.5 (34.1)	32.6 (34.8)	33.7 (35.5)
Amomum subulatum	30.4 (33.5)	32.9 (35.1)	30.0 (33.2)	18.2 (25.2)
	S. Ed	**CD (0.05%)**	**CD (0.01%)**	
Plant extract (P)	**1.7**	**3.4**	**4.4**	
Concentration (C)	**0.6**	**1.1**	**1.5**	
P X C	**3.4**	**6.7**	**8.9**	

*Figure in the parentheses represents arcsine transformed values

inhibition of 74.81 per cent was recorded with *S. nigrum* at 5 per cent concentration and was significantly superior over other treatments (Fig. 5). Similarly, *S. torvum* was also found to be inhibitory to the pathogen wherein, an inhibition of 65.56 per cent was observed at the highest concentration (Fig. 6). *A. indica* exhibited a maximum of 59.63 and 57.78 per cent inhibition at 2.5 and 5 per cent concentrations, respectively (Fig. 7). However, at higher concentrations (10% and 20%) the extract was not found effective compared to lower concentrations. *M. jalappa* at highest concentration of 20 per cent showed an inhibition of 58.82 per cent, but the lower concentrations were found to be less inhibitory to the pathogen. Among 35 plant species tested, phytoextracts from 10 plant species showed only less than 15 per cent growth inhibition at all concentrations tested. Extracts of *D. stramonium* was found to promote growth of the pathogen.

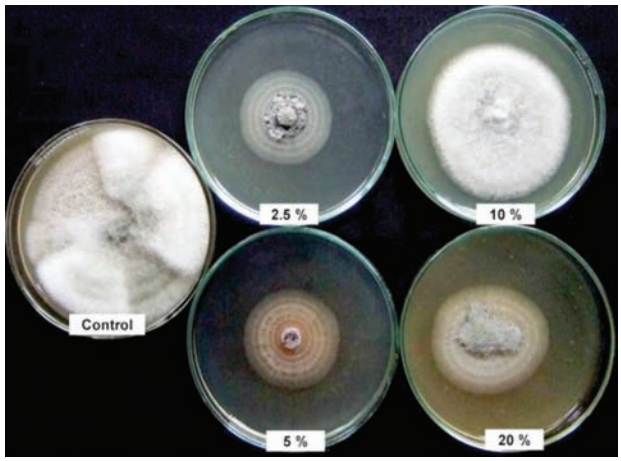

Fig. 4. ***In vitro*** **antifungal activity of** ***Azadirachta indica*** **extract on cardamom isolate of** ***Colletotrichum gloeosporioides***

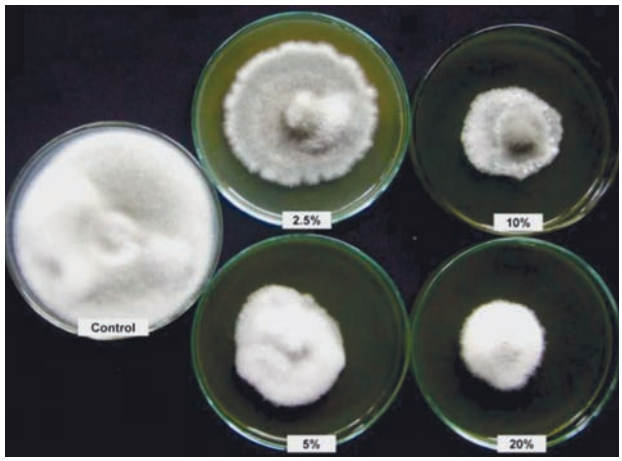

Fig. 6. ***In vitro*** **antifungal activity of** ***Solanum torvum*** **extract on black pepper isolate of** ***Colletotrichum gloeosporioides***

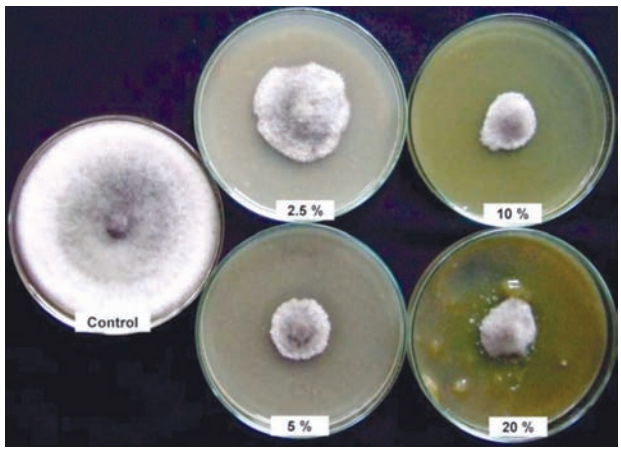

Fig. 5. ***In vitro*** **antifungal activity of** ***Solanum nigrum*** **extract on black pepper isolate of** ***Colletotrichum gloeosporioides***

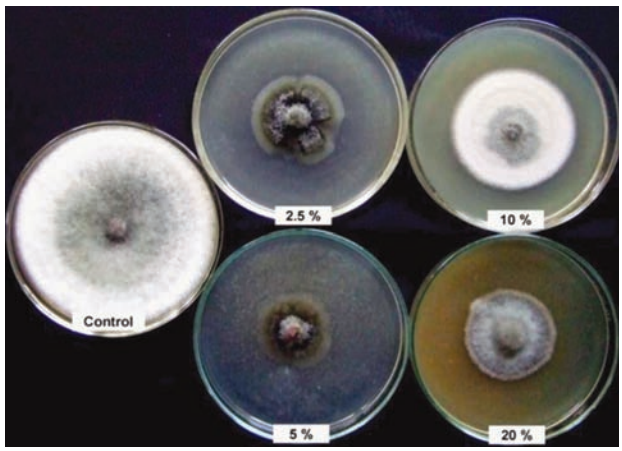

Fig. 7. ***In vitro*** **antifungal activity of** ***Azadirachta indica*** **extract on black pepper isolate of** ***Colletotrichum gloeosporioides***

On contrary to the plant species that exhibited growth promotional activity, species belonging to *Solanum* (*S. nigrum* and *S. torvum*) and *A. indica* were found to have profound inhibitory effect on growth of the pathogen. However, differential sensitivity could be observed in response to difference in concentrations of the phyto-extracts. Plant species belonging to the genus *Solanum* have been reported to have significant pharmacological activity (McKee, 1959). Steroidal alkaloid solanidine has been isolated from *S. nudum* and 23-S-23 hydroxyisolasodine from *S. panduraeforme* and solarmargine from *S. marginatum* (Babalola *et al*., 2012). Acetone, methanol and leaf extracts of *S. tomentosum* exhibited broad spectrum antibacterial and antimycotic activity and inhibited *Fusarium oxysporum* and *Aspergillus niger* under *in vitro* conditions (Aliero and Afolayan, 2006). Extracts of neem leaf, neem oil and seed kernels were reported to possess compounds with high antimycotic activity (Biswas *et al*., 2002). Govindachari *et al.* (1999) reported that, n-hexane fractions of leaf extracts of *A. indica* exhibited high antifungal activity as evidenced by inhibition of conidial germination of *F. oxysporum* and *C. lindemuthianum.*

Morphological malformations manifested on the hyphae of *C. gloeosporioides* in treatments with

S. nigrum, *S. torvum* and *A. indica* were revealed through microscopical examinations. The major morphological alterations noticed were increase in size and number of vacuoles in the hyphal strands (Fig. 8), thickening of hyphal wall (Fig. 9), anomaly in branching pattern (Fig. 10) and abnormal hyphal tip swelling (Fig. 11 a, b, c) whereas, no such alterations were noticed in the hyphae developed in control. The study indicated that, the extracts inhibited hyphal growth and resulted in irreversible morphological and structural alterations like abnormal swelling and excessive branching of the hyphae which would have restricted the colony growth *in vitro*. It may be inferred that, these secondary metabolites might have adversely affected various physiological/biochemical pathways by interacting with various enzymes or other moieties essential for growth, resulting in irreversible malformations induced on morphological architecture of the pathogens. Razzaghi-Abyaneh *et al*. (2005) observed deformation and vacuolation of mycelial protoplasm and herniation of cytoplasmic contents in *Aspergillus parasiticus* cultured on *A. indica* amended media. Bianchi *et al*. (1997) reported inhibition of mycelial development in *Rhizoctonia solani*, *C. lindemuthianum* and *Fusarium solani* with micronized garlic powder due to increased vacuolization, reduction in cytoplasmic contents, thickening of cell wall and accumulation of osmiophilic bodies. The hyphae of *F. oxysporum* f. sp. *Ttulipae* treated with *Allium fistulosum* exhibited

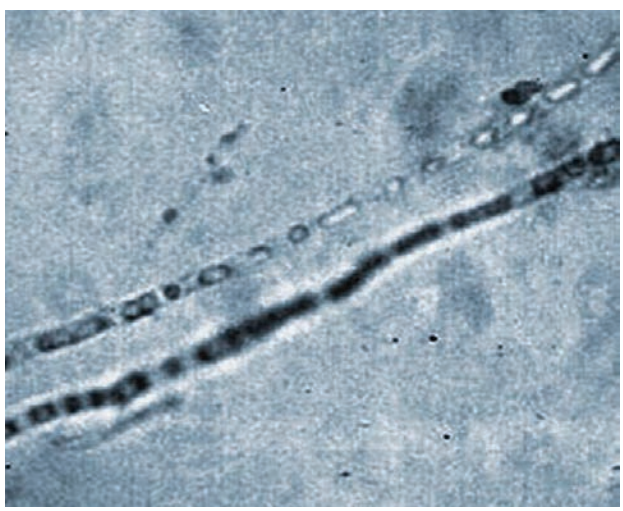

Fig. 8. Vacuolation of hyphal strands of *Colletotrichum gloeosporioides*

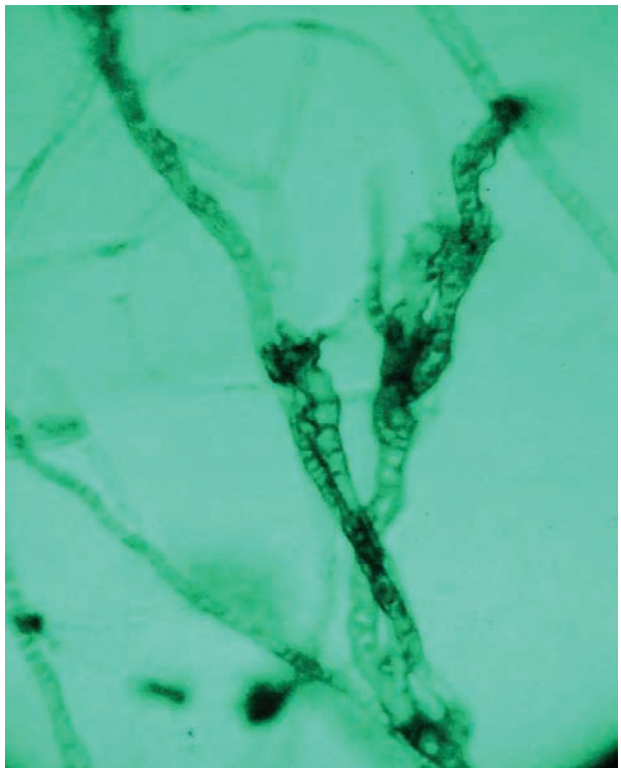

Fig. 9. Thickening of hyphal wall of *Colletotrichum gloeosporioides*

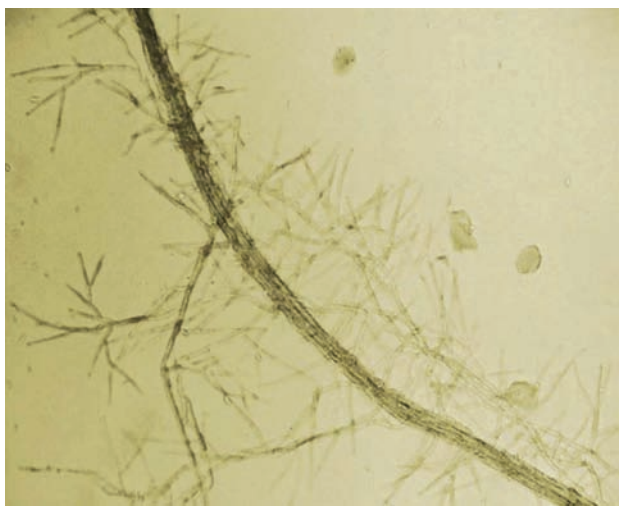

Fig. 10. Anomaly in hyphal branching pattern of *Colletotrichum gloeosporioides*

ultrastructural changes including destruction of organelles, degenerated cytoplasm and appearance of electron dense material in the hyphal cells (Parvu *et al.*, 2006). Rahman *et al*. (2007) observed alterations in *C. gloeosporioides* hyphae when co-cultured with *Bacillus cepacia* and *Pseudomonas*

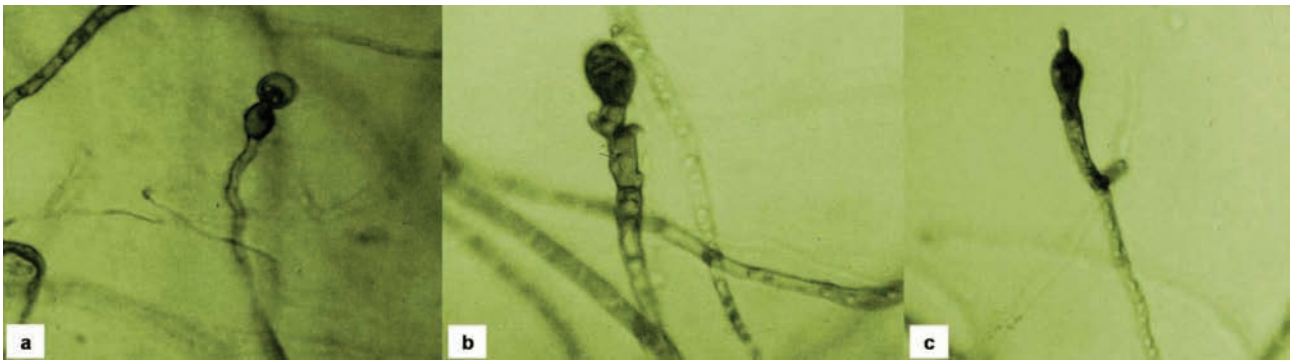

Fig. 11. Abnormal hyphal tip swellings in *Colletotrichum gloeosporioides*

aeruginosa which included, thickening of hyphal tips, malformation, vacuolation and swellings at hyphal tips.

C. gloeosporioides, the cosmopolitan hemi-biotrophic ascomycetous fungus is a major cause of concern to cardamom and black pepper industry. The pathogen endowed with an array of degradative enzymes and specialized strategies for host invasion, has the potential to create havocs in plantations, if adequate management measures are not formulated and executed timely. The pursuit for environmentally secure, economically viable and effective strategies for the management of plant diseases has led to an increased use of plant species and plant-based products hailed as 'green-pesticides' in agriculture (Gurjar *et al*., 2012). Plants have boundless ability to synthesize secondary metabolites of which, most belongs to the category of phenols or their oxygenated derivatives. These groups of compounds exhibits remarkable antimicrobial activity and perhaps, serves as integral components of host defense machinery against a broad spectrum of pathogenic invaders (Bajpai and Kang, 2012; Gurjar *et al*., 2012; Kumaran *et al*., 2013).

Development and use of natural antimicrobial pesticides would help to decrease the adverse impact of synthetic agents on delicate equilibrium of nature. In this respect, natural fungicides may be efficient, biodegradable, cost-effective and less toxic to the nature and food-agriculture based industries. The present study, threw light on the effectiveness of *S. nigrum*, *S. torvum* and *A. indica* in inhibiting growth of the pathogen under *in vitro* conditions and the possible mechanism of growth inhibitory activity. However, a thorough insight into the possible role played by the potential bio-active principles in dictating microbial-plant interactions is highly essential which, further contributes immensely to the process of bio-prospecting for mining the untapped molecules, possessing tremendous biological significance.

Acknowledgements

The authors thank The Director, ICAR-Indian Institute of Spices Research, Kozhikode and The Head, ICAR-IISR Regional Station, Appangala for providing facilities and Indian Council of Agricultural Research, New Delhi for financial support in the form of ALCOCERA, an Outreach Programme on Diagnosis and Management of Leaf Spot Diseases in Field and Horticultural Crops.

References

Alemu, K., Ayalew, A. and Weldetsadi, K. 2014. Evaluation of antifungal activity of botanicals for postharvest management of mango anthracnose (*Colletotrichum gloeosporioides*). *International Journal of Life Sciences* **8**:1-6.

Aliero, A.A. and Afolayan, A.J. 2006. Antimicrobial activity of *Solanum tomentosum*. *African Journal of Biotechnology* **5**: 369-372.

Babalola, I.T., Adelakun, E.A. and Garba, S.Y. 2012. Evaluation of antimicrobial activity of crude methanol extract of *Solanum noduliflorum* Jacq. (Solanaceae). *Journal of Pharmacognosy and Phytochemistry* **1**: 1-5.

Bajpai, V.K. and Kang, S.C. 2012. *In vitro* and *in vivo* inhibition of plant pathogenic fungi by essential oil and extracts of *Magnolia liliflora* Desr. *Journal of Agricultural Science and Technology* **14**: 845-856.

Bianchi, A., Zambonelli, A., D'Aulerio, A.Z. and Bellesia, F. 1997. Ultrastructural studies of the effect of *Allium sativum* on phytopathogenic fungi *in vitro*. *Plant Disease* **81**: 1241-1246.

Biju, C.N., Praveena, R., Ankegowda, S.J., Darshana, C.N. and Jashmi, K.C. 2013. Epidemiological studies on black pepper anthracnose caused by *Colletotrichum gloeosporioides*. *Indian Journal of Agricultural Sciences* **83**: 1199-1204.

Biswas, K., Chattopadhyay, I., Banerjee, R.K. and Bandopadhyay, U. 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science* **82**: 1336-1345.

Bonzi, S., Somda, I., Zida, E.P. and Sérémé, P. 2012. *In vitro* antifungal activity of various local plant extracts in the control of *Phoma sorghina* (Sacc.) and *Colletotrichum graminicola* (Ces.) Wilson, as sorghum seed mold pathogen in Burkina Faso. *Tropiculture* **30**: 103-106.

Chethana, C.S., Chowdappa, P., Pavani, K.V., Biju, C.N., Praveena, R. and Sujatha, A.M. 2015. Morphological and multi-loci gene analysis of five species of *Colletotrichum* responsible for anthracnose on black pepper in South India. *International Journal of Advanced Biotechnology and Research* **6**(3): 327-342.

Chethana, C.S., Chowdappa, P., Biju, C.N., Praveena, R. and Sujatha, A.M. 2016. Molecular and phenotypic characterization revealed six *Colletotrichum* species responsible for anthracnose disease of small cardamom in South India. *European Journal of Plant Pathology* **146**(3): 465-481.

Govindachari, T.R., Suresh, G. and Masilamani, S. 1999. Antifungal activity of *Azadirachta indica* leaf hexane extract. *Fitoterapia* **70**: 417-420.

Gurjar, M.S., Ali, S., Akhtar, M. and Singh, K.S. 2012. Efficacy of plant extracts in plant disease management. *Agricultural Sciences* **3**: 425-433.

Kumaran, R.S., Choi, Y.K., Jeon, H.J., Jung, H., Song, K.G. and Kim, H.J. 2013. *In vitro* antagonistic efficacy of plant extracts on the enzyme activity of *Colletotrichum gloeosporioides*. *African Journal of Microbiology Research* **7**: 1069-1076.

Masangwa, J.I.G., Aveling, T.A.S. and Kritzinger, Q. 2013. Screening of plant extracts for antifungal activities against *Colletotrichum* species of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp). *Journal of Agricultural Science* **151**: 482-491.

McKee, R.C. 1959. Factors affecting the toxicity of solanine and related alkaloids to *Fusarium caeruleum*. *Journal of General Microbiology* **20**: 686-696.

Nybe, E.V., Miniraj, N. and Peter, K.V. 2007. Introduction. In: *Spices. Horticulture Science Series - 5* (Ed). Peter, K. V. New India Publishing Agency, New Delhi, pp. 1-10.

Ogbebor, N.O., Adekunle, A.T. and Enobakhare, D.A. 2007. Inhibition of *Colletotrichum gloeosporioides* (Penz.) Sac. causal organism of rubber (*Hevea brasiliensis* Muell. Arg.) leaf spot using extracts. *African Journal of Biotechnology* **6**: 213-218.

Patel, K.D. and Joshi, K.R. 2001. Antagonistic effect of some bioagents *in vitro* against *Colletotrichum gloeosporioides* Penz. and Sacc. the causal agent of leaf spot of turmeric. *Journal of Mycology and Plant Pathology* **31**: 126.

Parvu, M., Tudoran, L.B., Casian, O.R., Vlase, L. and Tripon, S. 2006. Ultrastructural changes in *Fusarium oxysporum* f. sp. *tulipae* hyphae treated *in vitro* with *Allium fistulosum* plant extract. *Annals of RSCB* **15**: 65-72.

Praveena, R., Biju, C.N., Senthil Kumar, R., Darshana, C.N. and Jashmi, K.C. 2013. Preliminary evaluation of cardamom accessions against leaf blight/chenthal disease. *Indian Phytopathology* **66**: 112-113.

Rahman, M.A., Kadir, J., Mahmud, T.M.M., Rahman, R.A. and Begum, M.M. 2007. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gloeosporioides* in papaya. *Asian Journal of Plant Sciences* **6**: 12-20.

Ravindran, P.N. 2000. Introduction. In: *Black pepper - Piper nigrum.* (Ed). Ravindran P.N. Hardwood Academic Publishers, India, pp. 1-22.

Ravindran, P.N. 2002. Introduction. In: *Cardamom - The Genus Elettaria*. (Eds). Ravindran, P.N. and Madhusoodanan, K.J. Taylor and Francis, London and New York, pp. 1-10.

Razzaghi-Abyaneh, M., Allameh, A., Tiraihi, M., Shams-Ghahfarokhi, M. and Ghorbanian, M. 2005. Morphological alterations in toxigenic *Aspergillus parasiticus* exposed to neem (*Azadirachta indica*) leaf and seed aqueous extracts. *Mycopathologia* **159**: 565-570.

Shovan, L.R., Bhuiyan, M.K.A., Begum, J.A. and Pervez, Z. 2008. *In vitro* control of *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum*. *International Journal of Sustainable Crop Production* **3**: 10-17.

Tasiwal, V., Benagi, V.I., Hegde, Y.R., Kamanna, B.C. and Naik, K.R. 2009. *In vitro* evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. *Karnataka Journal of Agricultural Sciences* **22**: 803-806.

Thomas, J. and Bhai, R.S. 2002. Diseases of cardamom (fungal, bacterial and nematode diseases). In: *Cardamom-The Genus Elettaria.* (Eds). Ravindran, P.N. and Madhusoodanan, K.J. Taylor and Francis, London and New York, pp. 160-179.