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Antifungal efficacy of plant extracts, biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli

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Abstract

The present investigation was conducted during 2015-2017 to evaluate the effectiveness of extracts of Bavchi seeds (*Psoralea corylifolia*), Datura leaves (*Datura* sp.) and Ghaneri leaves (*Lantana camera*), biocontrol agents against *Colletotrichum capsici*. Among the *Pseudomonas fluorescens* and *Trichoderma viride* tested by dual culture technique. *Trichoderma viride* suppressed the growth of *Colletotrichum capsici* by 80.00%. The three botanicals viz., *Psoralea corylifolia*, *Datura* sp. and *Lantana camera* at different concentrations 250µl, 500µl, 750µl and 1000µl were tested by poisoned food technique. Methanolic extract of *Psoralea corylifolia* @ 1000µl showed the highest inhibition against *Colletotrichum capsici*. Treatment with *P. fluorescens* (culture filtrate @ 0.5%) + *T. viride* (culture filtrate @ 0.5%) + methanolic extract of *Psoralea corylifolia* @ 2% proved highly effective in reducing disease intensity (80%) in chilli under detached fruit bioassay.

Keywords: *Colletotrichum capsici*, *psoralea*, botanicals and antifungal efficacy etc.

Introduction

The antifungal compounds in plants play an important role in disease resistance (Mahadevan, 1982) [21]. Such compounds are selective of their toxicity, and play important role in plant disease management (Singh and Dwivedi, 1987) [32]. Several plant species also possess antifungal properties (Akinbode and Ikotun, 2008; Yasmin *et al.*, 2008) [1,38]. Medicinal plants viz., *Azadirachta indica*, *Ocimum basilicum*, *Datura stramonium*, *Tagetes erecta* and *Allium sativum* at 2, 4, 6 and 8% concentrations showed effective antifungal efficacy (Chohan *et al.*, 2011) [10].

Chilli is an important commercial crop grown in India. Although production is high in India. Among all the diseases, anthracnose disease is major constraint to chilli production worldwide resulting in high yield losses. This fungal disease caused by *Colletotrichum* species drastically reduces the quality and yield of fruit resulting in low returns to farmers. In India, in severe cases, pre harvest and post-harvest losses comprise up to more than 50%. Significant yield losses (Sahitya *et al.*, 2014) [30].

The *Psoralea corylifolia* is commonly known as babji, bakuchi and bavanchi. Is belongs to Fabaceae family. Bakuchi grows throughout India, *Psoralea corylifolia* Linn, has multifarious uses as it is an important component of Ayurveda. It is remedy as acts antifungal. In the present investigation it was found that phenols, alkaloids, tannins, flavonoids and saponin are present in seeds of plant. TLC and HPLC also confirmed these results. (Pandey *et al.*, 2013) [26]

Datura stramonium is a poisonous and wasteland weed, member of solanaceae family. It is also known as thorn apple. It contains toxic and pharmacological properties due to presence of many biologically active compounds such as alkaloids, atropine, scopolamine, tannin, carbohydrates and proteins. Traditionally it is used in many drugs for treatment of skin disorder, ear pain, cough and asthma. Ethanolic extract of *Datura stramonium* contains significant antifungal potential against some important plant pathogenic fungi and thus could be used as alternate of chemical fungicides for management of fungal infection in plants (Sharma *et al.*, 2014) [31].

Lantana camera is a significant weed of which there are some 650 varieties in over 60 countries. Allelopathy is the influence of one plant upon another plant growing in the vicinity by the release of certain metabolic toxic products in the environment. It covers biochemical interactions may harmful to fungi and bacteria (Mishra, 2015) [24].

Use of biocontrol agents in combination with reduced doses of chemical fungicide has recently been emphasized for sustainable agriculture (Andrabi *et al.*, 2011) [4]. Cultural methods of disease management are not sufficiently effective against the pathogens having prolonged

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saprophytic survival ability. The use of *Trichoderma* has attracted attention because of its efficacy against wide range of plant pathogens and for its growth promoting action (Harman *et al.*, 2004) [15]. The *Trichoderma* species have been reported quite effective against chickpea wilt under glasshouse and field conditions. Selected isolates of *P. fluorescens* have also been found suppressive against chickpea wilt.

Weeds are the plants, which grow where they are not wanted. Weeds can also be referred to as plants out of place. Weeds are unwanted or undesirable plants compete with crops for water, soil nutrients, light and space (*i.e.* CO₂) and thus reduce crop yields. Weeds are competitive and adaptable to all the adverse environments. It has been estimated that in general weeds cause 5% loss to Agricultural production in most developed countries. 10% loss in less developed countries and 25% loss in least developed countries. Reduction of crop yield has a direct correlation with weed competition. Weeds compete for water, light, nutrients and space. Weeds compete for water in dry land and for nutrients in irrigated crops. It includes reduction in crop yields and production efficiency and erosion of crop quality. Reduction in crop yields and production efficiency is direct effect due to weeds. It varies from 34.3% to 89.8% depending upon the 7 crop. In rice (30-35%), wheat (15-30%), Maize, sorghum, pulses, oilseeds (18-85%), sugarcane (38.8%), cotton (47.5%), sugar beet (48.4%) and onion (90.7%). Beside the direct reduction in crop yields there are many indirect ways by which the weeds may be troublesome in agriculture. For example in weedy fields management practices become cumbersome. Certain weeds cause sickness and death of animals due to high levels of alkaloids, tannins, oxalates, glucosides or nitrates.

The present investigation is an attempt to explore the possibilities for utilizing such huge biological waste along with biocontrol agents for management of anthracnose of chilli. The present investigation is therefore, undertaken to test the efficacy of common weed extracts against *Colletotrichum capsici*.

Materials and Methods

An experiment was conducted to check the efficacy of different plant solvent extracts and biocontrol agents against *Colletotrichum capsici* during years 2015-17. Weeds viz., *Datura sp.* and *Lantana camera* were collected from fields of Dr. PDKV Akola whereas *Psoralea corylifolia* seeds were procured from Akola city of Maharashtra. Biocontrol agent's viz., *T. viride*, and *P. fluorescens* were obtained from Department of Plant Pathology, Dr. P.D.K.V., Akola. Mature red chillies genotype K1 were used as a susceptible host for *Colletotrichum capsici* throughout the experiment.

Pathogenicity of *Colletotrichum capsici* on detached chilli fruit

The pathogenicity test *Colletotrichum capsici* was performed on most susceptible chilli genotype (K1) using plug inoculation method following modified protocol by Prior to inoculation, chilli fruits were surface sterilized with 70 percent (v/v) ethanol and air dried in the laboratory for 3-4 minutes. Prior to inoculations, one set of fruit was given an injury with the help of sterilized testing needle (Thind and Jhooty, 1990) [34] and another set kept uninjured. Inoculation was made by placing a fungal disc (5 mm) taken from a young fungal colony of *C. capsici* on the wound. The fruits inoculated by placing only a potato dextrose agar disc on the

wound served as control. The inoculated fruits were kept in moist chamber at room temperature (25-28 °C) for ten days. The pathogenicity test was closely monitored for symptom development. Re-isolation of pathogen from artificially inoculated fruits were carried out and resultant cultures compared with original inoculants to satisfy Koch's postulates.

Preparation of plant solvent extracts

All leaves of *Psoralea corylifolia*, *Datura sp.* and *Lantana camera* were thoroughly rinsed with tap water to remove impurities; dried separately under the shade with occasional up and down mixing for 4 weeks. The dried leaves were powdered with the grinder and stored in airtight container until further use (Thenmozhi *et al.*, 2011) [33]. Acetone, ethanol, methanol and chloroform were used as solvent for preparing crude extracts. Forty gram powder of each weed was soaked in 200 ml of each solvent in 500 ml conical flask cotton plugged tightly and wrapped with paper; kept on the rotary shaker at 150 rpm and at 30 °C temperature for four days and then allowed to stand for 5 hr to settle the leaves debris. Supernatant from each flask was filtered separately through Whatman No. 1 filter paper and evaporated at room temperature (30-35 °C) up to complete removal of the solvent. Residual leaves were re-extracted thrice to harvest maximum metabolites. Concentrated air dried extracts were weighed separately and transferred into small vials and kept in the refrigerator at 5 °C for further use. The resultant crude extracts were used at four different concentrations for growth inhibition assay against *Colletotrichum capsici*.

In vitro evaluation of plant solvent extracts by poisoned food technique on PDA medium

The efficacy of acetone, ethanol, methanol and chloroform extracts of *Psoralea corylifolia*, *Datura sp.* and *Lantana camera* at 250, 500, 750 and 1000 ul concentrations were tested against *Colletotrichum capsici* under *in vitro* condition following poisoned food technique on PDA medium (Al-Rahmah *et al.*, 2013) [2].

One gram crude extract of all the plants extracted with acetone, ethanol, methanol and chloroform were diluted in 10 ml DMSO separately and from this 250, 500, 750 and 1000 ul suspension were poured separately in conical flasks which containing 60 ml sterilized melted PDA medium for 3 plates. The conical flasks were shaken well for uniform mixing of plant extract with media and poured in plates then allowed for solidification. In control set, only 250, 500, 750 and 1000 ul DMSO were used. For each treatment, 3 replicates (plates) were used. All the plates were inoculated individually with 5 mm diameter discs of the test fungal culture and then incubated at 28±2 °C, until the control plates reached full growth. To know the effect of different plant extracts. The percent growth inhibition (I) of test fungus was calculated by using formula suggested by Vincent (1947).

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of fungus in control in mm

T = Growth of fungus in treatment in mm

In vitro evaluation of bioagents by dual culture assay

Antagonistic activity of *Pseudomonas fluorescens* and *Trichoderma viride* on growth of *Colletotrichum capsici* was

studied by dual culture technique on PDA plates. Inoculum disc of 5 mm diameter was taken from 7 days old culture of *Colletotrichum capsici*. These discs were placed at center of respective PDA plates. Then bacterial antagonists were streaked parallel on both sides of fungal pathogens leaving 3 cm distance between them. In case of fungal antagonist each plate was inoculated with 5 mm mycelial disc of fungal pathogen and *Trichoderma viride* were placed at side by side on medium in each plate approximately at a distance of 4 cm away from each other. Similarly one set of fungus without any bioagent culture served as control.

The inoculated plates were incubated at 28 ± 2 °C for 7 days. Colony diameter of test fungus in each plate was measured and mean diameter and percent inhibition was calculated by using formula suggested by Vincent (1947) [36].

Preparation of culture filtrate of *T. viride* and *P. fluorescens*

The microbial bioagents which were used in this study were *Trichoderma viride* and *Pseudomonas fluorescens*. The fungal bioagent which we used was cultured on potato dextrose broth (PDB) for 15 days at 20–25 °C. Then, the fungal biomass was centrifuged at 10000 rpm for 20 min, and the culture medium was discarded. Next, the supernatant was filtered by passing the culture broth through a sterile membrane filter (0.2 µm). *Pseudomonas fluorescens* was cultured on Kings-B medium in 250-ml Erlenmeyer flasks on a rotary shaker at 150 rpm at 28–30 °C. After 24 h, bacterial cell suspension was pelleted by centrifugation at 7000 rpm for 10 min. Then the supernatant was filtered using a glass filter to obtain cell-free culture filtrate (El-Boghdady 1993) [14].

Preparation of pathogen inoculum

The inoculum of *Colletotrichum capsici* was prepared from twenty day old PDA grown culture in petriplates. Distilled sterile water was poured in the petriplates containing sporulating fungus and with the help of spatula, scraped the fungal colony and washed the plates 3-4 times to harvest all the spores. To get homogenous suspension, the sterile water containing spores and mycelial bits were finally strained through muslin cloth to get spore suspension having 10^7 spores/ml using a haemocytometer.

Control of anthracnose disease on chilli fruit by detached fruit bioassay

An experiment was conducted to determine the individual and combined effect of *P. fluorescens*, *T. viride* and methanolic extract of *Psoralea corylifolia* on Fruit rot of chilli during year 2016-2017. The culture filtrates of *T. viride* and *P. fluorescens* were prepared by following the method described by El-Boghdady (1993) [14] and Mishra *et al.*, (2011) [25]. Mature chilli fruits were surface sterilized in 70% alcohol for five minutes before being washed several times with sterile distilled water and then blotted dry on sterilized filter paper. The sterilized chilli fruits were pin pricked gently with a sterilized needle prior to inoculation. Four pepper fruits were placed on the three-layered sterile water-saturated blotter paper in the plastic petri plates, and four drops of the conidial suspension (10^7 conidia/ml, 15 µl for each drop) was inoculated on the pin-wounded (1 mm in depth) pepper fruit surface. Methanolic extract of *Psoralea corylifolia* and culture filtrate of bioagents were applied to a pin point wound on the mature chilli fruits one day before the conidia suspensions of the pathogen were applied. The inoculated fruits were incubated in a moist chamber and kept at room temperature.

The disease severity was observed daily for seven days (Jaihan *et al.*, 2016) [17].

The disease intensity was recorded on fruits in each treatment following the score chart 0 to 9 scale followed by Mayee and Datar (1986) [22]. The percent disease index (PDI) was calculated using McKinney (1923) [23] infection index.

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of fruit observed}} \times \frac{100}{\text{Maximum category value}}$$

This experiment included the following ten treatments: T1- *P. fluorescens* alone (culture filtrate @ 0.5%), T2- *T. viride* alone (culture filtrate @ 0.5%), T3- Methanolic extract of *Psoralea corylifolia* alone @ 0.5%, T4- Methanolic extract of *Psoralea corylifolia* alone @ 1%, T5- Methanolic extract of *Psoralea corylifolia* alone @ 2%, T6- *P. fluorescens* (culture filtrate @ 0.5%) + *T. viride* (culture filtrate @ 0.5%), T7- *P. fluorescens* (culture filtrate @ 0.5%) + methanolic extract of *Psoralea corylifolia* @ 2%, T8- *T. viride* (culture filtrate @ 0.5%) + Methanolic extract of *Psoralea corylifolia* @ 2%, T9- *P. fluorescens* (culture filtrate @ 0.5%) + *T. viride* (culture filtrate @ 0.5%) + methanolic extract of *Psoralea corylifolia* @ 2% and T10 Control (*Colletotrichum capsici* inoculated).

Experimental design and statistical analysis

An experiment of *in vitro* effect of solvent extracts on test pathogen was carried out by using completely randomized block design (FRBD) with three factors. Detached chilli fruit experiment was carried out by using completely randomized design and each treatment had three replications. ‘F’ test of significance was used to identify significant treatments. The standard error (SE) and critical difference (CD) at 1% level of probability were calculated.

Results and Discussion

The pathogenicity test of *Colletotrichum capsici* was determined on the injured detached fruits of chilli (Cv.K1), using pin prick method as described in “material and methods”. The fungus produced initial symptoms of the disease between 5 to 7 days on injured fruits. The morphological characters of the isolated pathogen were found to be similar to those of original culture. Hence, it was confirmed that, the fungus *C. capsici* was responsible for causing anthracnose and fruit rot of chilli and conforming the Koch’s postulates. However no such symptoms develop on un-injured fruit even after 12 days of inoculation. Vanan *et al.* (2005) [35] also proved and confirmed the pathogenic nature of *Colletotrichum capsici* in chilli. Similarly, pathogenicity was also proved by artificial inoculation in past on chilli fruit. (Datar, 1995 and Parey *et al.*, 2013) [12, 27].

In vitro evaluation of plant solvent extracts against *Colletotrichum capsici*

In vitro evaluation of plant solvent extracts revealed that all tested treatments of extracts had the considerable inhibitory effect on the growth of *Colletotrichum capsici*. Results of Table 1, represent that, at 1000 µl concentration (C4) 96.99% inhibition of mycelial growth of test fungus was recorded in S3P1 (methanolic extract of *Psoralea corylifolia*), followed by S2P1 (ethanolic extract of *Psoralea corylifolia*), S1P1 (acetone extract of *Psoralea corylifolia*). Lowest inhibition (64.61%) was recorded in S4P3 (chloroform extract of *Lantana camera*) interaction. Earlier study of Johnny *et al.* (2011) [18] suggested that Crude extract of *P. betle* in methanol

inhibited 85.25% of radial growth of *C. capsici*. Whereas, petroleum ether extract of seeds of *Psoralea corylifolia* was recorded a maximum antifungal activity (93.5%) in

Aspergillus flavus oryzae (Kiran *et al.*, 2011; Bhardwaj and Sahu 2014)^[20, 7].

Table 1: Effect of interaction mean of solvents x plants x concentrations (S x P x C) on growth of *Colletotrichum capsici*

S x P x C (Solvent x plant x Conc.)	Mycelial growth (mm)				% inhibition over control			
	C1	C2	C3	C4	C1	C2	C3	C4
S 1 P 1	59.60	52.38	22.07	11.43	33.76 (35.52)*	41.79 (40.27)	74.86 (59.91)	85.69 (67.78)
S 1 P 2	70.70	61.34	42.50	19.51	21.43 (27.57)	31.84 (34.35)	51.61 (45.92)	75.60 (60.40)
S 1 P 3	76.73	60.57	45.14	22.12	14.74 (22.57)	32.69 (34.87)	48.59 (44.19)	67.91 (58.33)
S 2 P 1	55.34	39.07	18.50	6.17	38.50 (38.35)	56.61 (48.78)	78.93 (62.67)	92.27 (73.86)
S 2 P 2	63.34	55.22	32.35	12.99	29.71 (32.97)	38.63 (38.43)	63.15 (52.64)	83.76 (66.24)
S 2 P 3	68.85	59.73	42.44	28.17	23.49 (28.99)	33.63 (35.44)	51.67 (45.96)	64.77 (53.53)
S 3 P 1	46.86	18.43	7.13	2.40	47.92 (43.80)	79.52 (63.09)	91.87 (73.50)	96.99 (80.06)
S 3 P 2	55.63	44.77	20.69	13.93	38.18 (38.16)	50.24 (45.13)	76.44 (60.96)	82.58 (65.33)
S 3 P 3	73.10	65.30	35.34	20.44	18.76 (25.67)	27.43 (31.58)	59.76 (50.63)	74.44 (59.63)
S 4 P 1	61.87	56.35	28.80	17.41	31.24 (33.98)	37.37 (37.68)	67.20 (55.06)	77.56 (61.73)
S 4 P 2	76.10	59.73	41.40	19.79	15.43 (23.13)	33.63 (35.44)	52.86 (46.64)	75.25 (60.16)
S 4 P 3	80.53	69.50	50.42	28.30	10.51 (18.91)	22.77 (28.50)	42.58 (40.73)	64.61 (53.50)*
Control	90.00	90.00	87.83	80.00	0.00	0.00	0.00	0.00
F Test	Sig.				Sig.			
S.E (M)±	0.28				0.25			
C.D. at (p=0.01)	1.04				0.95			

*Figures in parenthesis are arc sin transformed values

Average of three replications

Solvents(S): S1- acetone, S2- ethanol, S3- methanol and S4- chloroform

Plants (P): P1- *Psoralea corylifolia*, P2- *Datura* sp. and P3- *Lantana camera*

Concentrations(C): C1- 250µl, C2-500µl, C3- 750µl and C4- 1000µl

The results are also in lined with the results of Kamber *et al.* (2014)^[19] showed highest inhibition (74.19%) of *C. capsici* in methanolic extract of *M. indica*. Rajput and Palakshappa (2014)^[29] also reported neem based formulations were effective against *C. capsici*. Prasad and Anamika (2015)^[28] found that ethanol extract of *Lantana camera* possess significant fungicidal effect on growth of *C. gloeosporioides*. Begum and Nath (2015)^[6] tested efficacy of four botanical oils *viz.*, Garlic, Neem, Polyalthia and Citronella at different concentrations and inhibited growth of *C. capsici*. Marigold and *gaillardia* extracts effectively suppressed growth (81.59%) of *F oxysporum* f. sp. *ciceri* (Wavare *et al.* 2017)^[37].

Evaluation of biocontrol agents against *Colletotrichum capsici* by dual culture technique

Comparison was made between two bioagents for their ability to control mycelial growth of *Colletotrichum capsici* by dual culture technique. All the bioagents were effective in reducing fungal growth. Among two bioagents tested, maximum inhibition of mycelial growth was noticed in *Trichoderma viride* (80.48%) and was found to be significantly superior over other treatments

Table 2: Efficacy of bioagents on mycelial growth of *Colletotrichum capsici*

Tr. No.	Treatment	Radial mycelial growth (mm)	Percent inhibition
1.	<i>T. viride</i>	17.56	80.48(63.80)*
2.	<i>P. fluorescens</i>	31.10	65.44(53.99)*
3.	Control	90.00	00.00
	F test	Sig.	Sig.
	S.E. (M)±	0.91	0.85
	C.D. at (p=0.01)	3.78	3.53

*Figures in parenthesis are arc sin transformed values

Average of five replications

Similar results were observed by Bilal *et al.* (2010)^[8] who showed that three bioagents (*Trichoderma viride*, *T. harzianum* and *Gliocladium virens*) were evaluated against *Colletotrichum lindemuthianum*. Maximum inhibition being with *T. viride* (69.21%). Chacko and Gokulapalan (2014)^[9] reported *T. viride* caused mycelia growth inhibition of 55.5% in dual culture. The formation of hyphal coils by *T. viride* on pathogenic colonies was also noticed. *Pseudomonas fluorescens* showed 90% of the radial growth inhibition of the test pathogen *Colletotrichum capsici*. Azad *et al.* (2013)^[5] found that *Trichoderma viride* gives 77.60% growth inhibition against *C. gloeosporioides*. Salma Begum and Nath

(2015)^[6] tested *T. harzianum* isolate Th-2 was found most effective giving 77.78%, 100%, 83.33% and 88.89% inhibition on the mycelial growth of SCC1, SCC2, SCC3 and SCC4 respectively.

Evaluation of methanolic extract of *Psoralea corylifolia* and bioagents against *Colletotrichum capsici* causing anthracnose of chilli under detached chilli fruit bioassay

Two potential bio-control agents and methanolic crude extract of *Psoralea corylifolia* with three different concentrations alone and in combination were screened against fruit rot of chilli caused by *Colletotrichum capsici* on detached fruit

technique. Results from Table 6 and plate 7 revealed that, among all the treatments, maximum 81.41% disease reduction over control was observed in treatment T9-*P. fluorescens* (culture filtrate @ 0.5%) + *T. viride* (culture filtrate @ 0.5%) +

methanolic extract of *Psoralea corylifolia* @ 2% followed by 70.93% in T8- *T. viride* (culture filtrate @ 0.5%) + methanolic extract of *Psoralea corylifolia* 2%). Lowest disease reduction was reported in T1 (50.01%).

Table 3: Effect of *Pseudomonas fluorescens*, *Trichoderma viride* and methanolic extract of *Psoralea corylifolia* alone and in combination on fruit rot of chilli caused by *Colletotrichum capsici* on detached chilli fruit

Tr. No.	Treatment	Fruit rot	
		Disease intensity (%)	Percent reduction over control
T1	<i>P. fluorescens</i> alone (culture filtrate @ 0.5%)	39.80 (39.11)*	50.01
T2	<i>T. viride</i> alone (culture filtrate @ 0.5%)	37.02 (37.47)	53.50
T3	Methanolic extract of <i>Psoralea corylifolia</i> alone @ 0.5%	34.25 (35.81)	56.98
T4	Methanolic extract of <i>Psoralea corylifolia</i> alone @ 1%	29.62 (32.95)	62.79
T5	Methanolic extract of <i>Psoralea corylifolia</i> alone @ 2%	29.40 (32.01)	63.07
T6	<i>P. fluorescens</i> (culture filtrate @ 0.5%) + <i>T. viride</i> (culture filtrate @ 0.5%)	25.91 (30.59)	67.45
T7	<i>P. fluorescens</i> (culture filtrate @ 0.5%) + methanolic extract of <i>Psoralea corylifolia</i> @ 2%	26.84 (31.19)	66.28
T8	<i>T. viride</i> (culture filtrate @ 0.5%) + Methanolic extract of <i>Psoralea corylifolia</i> @ 2%	23.14 (28.74)	70.93
T9	<i>P. fluorescens</i> (culture filtrate @ 0.5%) + <i>T. viride</i> (culture filtrate @ 0.5%) + methanolic extract of <i>Psoralea corylifolia</i> @ 2%	14.80 (22.61)	81.41
T10	Control	79.62 (63.21)	
	F test	Sig.	
	S.E. (M)±	0.77	
	C.D. at (p=0.01)	3.12	

Figures in parenthesis are arc sin transformed values
Average of three replications

The results are in agreement with earlier study of Hong *et al.* (2015) [16] who reported plant essential oils were reduced lesion diameter on the *C. gloeosporioides*-inoculated immature green pepper fruits compared to the inoculated control fruits. Showed 2% *Azadirachta indica* and 4% citric extract reduced post-harvest anthracnose of banana fruits. Garlic mallow and ginger extract effectively reduced disease severity on bell pepper fruit (Alves *et al.*, 2015) [3]. Jaihan *et al.* (2016) [17] also found that culture filtrate of entomopathogenic fungi inhibites growth of *C. capsici* and effectively reduced the disease severity on chilli fruits. Deressa *et al.* (2015) [13] reported that *P. juliflora* and *L. camera* leaf extract effectively reduced mango anthracnose.

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