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INTEGRATED MANAGEMENT OF ANTHRACNOSE OF *Capsicum annuum* L.

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Anthracnose on pepper fruit was first identified more than a century ago and poses a threat in most regions where bell pepper is grown. Anthracnose caused by *Colletotrichum capsici*. Anthracnose causes extensive losses in plants of the genus *Capsicum* before and after harvest especially in hot and humid climates. The current study was conducted to check the effect of plant extracts and some fungicide against anthracnose fruit rot of bell pepper. We have assessed three distinctive plant removes acquired from Eucalyptus, Dodonaea and Garlic as a biological control for the conceivable nearness of fungi toxic action against *C. capsici* by utilizing agar well diffusion procedure and also use a fungicide Mancozeb by applying food poison technique. Over all, the extract of Eucalyptus was found the most effective with the maximum inhibition zone of 3.16 cm at 150ul (75g/L), Showed with the inhibition zone followed by Dodonaea 2.83cm at 150ul (75g/L) concentration. Garlic was the third effective plant extract which was the least effective against *Colletotrichum capsici* which showed 1.70cm at 150ul (75g/L) zone of inhibition. Mancozeb showed the best result and it reduced the 71.7% growth of *Colletotrichum capsici* at 300 ppm respectively. The second most valuable concentration is 200ppm it reduced the 67.73% growth of *Colletotrichum capsici*. The 100ppm concentration of Mancozeb showed least effective result.
Keywords: Integrated management**,** Plant Extracts, Fungicide, Biological control.

INTRODUCTION

Capsicum annuum L. (Bell pepper) belongs to the family *Solanaceae* is an important constituent of many foods adding flavour, colour and is rich source of Vitamin 'C'. It is also well known for its nutritional properties (Ajith and lakshmidevi, 2010). Only recently a variety of sweet and hot paprika has been cultivated profusely and in view of its good profitability production and export possibilities has started to expand. Currently production is concentrated with 656, 532, 412 and 349 ha, respectively corresponding to 79% of the cultivated area nationwide during the period 2000-2009 the highest production was obtained in 2002 reaching 54796, grown in 2480 ha (Casilimas *et al.,* 2012). Anthracnose on pepper fruit was first identified more than a century ago and poses a threat in most regions where pepper is grown. Anthracnose caused by *Colletotrichum capsici* causes lesions on fruit that appear as sunken necrotic lesions that frequently coalesce and affected fruit are non-marketable. Early anthracnose was proposed as a common name for the disease that occurs on immature (green) bell pepper fruit to distinguish it from 'ripe-rot' anthracnose (Harp *et al*., 2008). Anthracnose causes extensive losses in plants of the genus *Capsicum* before and after harvest especially in hot and humid climates (Pereira *et al.,* 2011; Park *et al.,* 2012). The disease is considered of complex etiology since it can be caused by different species of *Colletotrichum*, including *C. acutatum*, *C. capsici* (Than *et al.,* 2008; Mahasuk *et al.,* 2009; Mongkolporn *et al.,* 2010; Park *et al.,* 2012). Synthetic fungicides are presently used as the primary approach for the control of plants diseases which are used at pre-harvest or post-harvest applications appearing as the main approach to reduce losses which are due to anthracnose. But uses of these chemicals are restricted due to the environmental concern due to their applications as alone, mixture or apply in sequence. Plant extracts are used now these days for the control of many diseases due to their non-toxicity, environmental friendly nature and their availability (Aqil *et al.,* 2010). Many plant extracts have potential as antimicrobial agents which can be applied to food and agriculture products because these extracts contain antimicrobial effects on microorganisms. These extracts are of Generally Recognized As Safe (GRAS) status. This study was conducted to check the antifungal properties of three plant extracts and a fungicide against *Colletotrichum capsici*. The comparative study of their activities at different doses and days was done.

MATERIALS AND METHODS

***Isolation of the Pathogen L*:** Many different types of media used for the isolation of pathogenic fungi associated which were associated with bell pepper anthracnose. PDA media was used in this research. Sections of 3-5 mm^2 were cut from the margin of the infected lesions and sterilized for one minute

in 2.0% sodium hypochlorite solution and rinsed in three changes of sterile distilled water (SDW). The sterile pieces were blotted dry using sterile filter papers and placed on Potato Dextrose Agar (PDA) in 9cm Petri dishes. The dishes were incubated at ambient conditions of light and temperature (25 ± 2°C) for 5 days after which cultures with salmon-pink sporulation typical of *Colletotrichum capsici* were sub cultured to obtain pure cultures, preserved in silica gel. It was revived by plating on PDA media.

***Preparation of spore suspension*:** Ten days old actively growing fungal plates were used for the preparation of spore suspension. Fungal mycelium along with spores was harvested by surface flooded of distilled water and then gentle scraping of fungus with the help of sterilized glass slide. The culture suspension was centrifuged at 4000 rpm for 2 minutes and then filtered. Plate dilution method was used to adjust the concentration of spore suspension. Distilled water was added to the suspension at concentration of 1×10^6 colony forming unit (CFU)/ ml (El- Badawy and Abdelgaleil, 2014).

***Use of Plants Parts/Materials/Extracts as a biological control*:** Leaves of 2 plants (Eucalyptus, Dodonaea) and bulb of 1 plant (Garlic) with known antifungal characteristics were selected for the *in-vitro* inhibition activity against *Colletotrichum capsici* pathogen (Table 1). Plant parts were washed thoroughly 2-3 times with running tap water, then with sterile distilled water, dried under shade at room temperature. Then the plants Parts were cut into small pieces before grinding and powdered with the help of electric grinder into fine powder.

***Plant Extract Preparation*:** Plant leaves were washed with tap water and dried. Plant leaves about 10g were put in to flat bottom flasks to which 100 mL of acetone or ethanol were added. After adding the solvent the flasks were labeled. After labeling, the flasks were covered with aluminum foil. Flasks were shook for 5 minutes to homogenize the solute and the solvent. Then the flasks were left for constant shaking in an incubator shaker (New Bronswick Scientific Exceler E24 incubator shaker series) at 250 rpm at 35ºC for 72 hours. After that, the mixtures of acetone or ethanol were filtered with the help of filter paper while aqueous mixtures were firstly centrifuged at 4000 rpm for 10 minutes into centrifuge (Centurion Scientific K3 Series) to get clear extract and then filtered. Samples were defused with 10mL of ethanol. Resulting extracts were taking as 100% concentration of plant extract.

***Agar Well Diffusion Method*:** To examine the antifungal potential activity of plants extracts, agar well diffusion method was used (Perez *et al.*, 1990). 1ml spore suspension ($1x10^6$spores/ml) was poured into sterilized plates then media was poured and homogenized. Wells of 5mm were made by using sterile cork borer on solidified PDA media. 50µl (25g/L), 100 µl (50 g/L), 150µl (75g/L) of acetone, ethanol and water extracts was poured into well. Three replicates were done for each concentration of the different extracts. In control plates the wells were added with sterilized distilled water. The plates were placed at room temperature for one hour so that the extract was pre-diffused into the medium (Esimone *et al.*, 1998). Then these plates were incubated at 28oC±2 for 3-4 days / till the culture in control is filled. After incubation the diameter of zone of inhibition around each well was measured.

***Fungicide Used against Colletotrichum capsica*:** To find out the efficacy and effective dose of the fungicides against the pathogen, poison food technique was used. Mancozeb were used to check the fungicidal effect on the fungal growth at three concentrations (100ppm, 200ppm, 300ppm) to observe the efficacy against the fungal growth. Fungicides at different concentrations of 100ppm, 200ppm and 300 ppm were mixed in PDA agar medium and poured in to Petri dishes. The inoculation was performed using small pieces of 72 hour old culture of *Colletotrichum capsici*. The inoculated petri dishes were randomized in three replicates, incubated at 25°C until the fungus acquired a 9 cm radial mycelia growth in control treatment. Petri dishes with only PDA medium were reserved as control. The efficacy of the various fungicides was examined against the radial mycelial growth of the pathogen.

Table 1: Plants Extract for antifungal activity of *Colletotrichum capsici*

Sr. No	Local Name	Botanical Name	Family	Plant Part
1	Dodonaea	*Dodonaea viscosa*	Sapindaceae	Leaves
2	Garlic	*Allium sativum*	Liliaceae	Bulbs
3	Eucalyptus	*Eucalyptus globulus*	Myrtaceae	Leaves

RESULTS AND DISCUSSION

The current study was conducted to check the effect of plant extracts and some fungicide against anthracnose (fruit rot) of bell pepper. Antimicrobial activity of plant extracts has been studied against different type of fungi for years but in a more intensified way in the last few decades. Many plant extracts have potential as natural antimicrobial agents that can be applied to agriculture produces, foods and pharmaceutics (Horburg, 1998). In this study, antifungal activity of three different plant extracts were evaluated. Extracted with methanol as extraction solvent had been checked against growth of the *Colletotrichum capsici* on capsicum fruit in laboratory conditions at constant temperature of 25^0C. From the selected 3 different plant extracts each extract was applied at three different doses (10, 25 and 50µL) and showed different antifungal properties. Antifungal activity of different plant extracts were screened against *Colletotrichum capsici* by using agar well diffusion method. Different concentrations of plant extracts were prepared in acetone, 80µL of each extracts were added in to the wells pieces from fully grown fungal culture were placed at the centre of each petri plate. Plates were incubated and inhibition zone were recorded after

5 and 10 days. All plant extracts showed different response against the growth inhibition of *Colletotrichum capsici* at different doses in contrast to control group, effect of each extract was analyzed separately. Eucalyptus showed most effective results in comparison with other two plant extracts and at second *Dodonaea viscosa* showed good antifungal response as compared to other one remaining extract of garlic was the least effective plant extract (fig 1). In this study which shows growth inhibition of pathogen at different doses and most effective results were obtained at dose of 50µL than other two doses of 10µL and 25 µL. While garlic extract showed least growth inhibition of the pathogen. It can be concluded that Eucalyptus and *Dodonaea viscosa* can be used as bio fungicides for the control of anthracnose of capsicum on capsicum fruits and will be safe to use (Table 2). Different concentrations (25% 50% and 75%) were prepared in acetone. Out of 3 plant extracts, *Eucalyptus* was found effective in inhibiting the mycelial growth of the pathogen when compared with control which exhibited no inhibition. *Eucalyptus* was the best plant extract which showed maximum inhibition zone after 5 to 10 days were (3.1 to 3.95 cm) respectively zone of inhibition at 75% concentration (Fig.1) and *Dodonaea viscose* was the 2nd aqueous plant extract which showed best result with 2.63 cm zone of inhibition at 75% concentration (Fig 1). Whereas, as very less inhibition was estimated by garlic extract at any concentration (Table 2). The mycelial growth of *Colletotrichum capsici* was reduced due to effectiveness of the test fungicide. Mancozeb at their different concentration inhibited the mycelial growth of *Colletotrichum capsici*. Mancozeb however, showed the best result and it reduced the 71.7% growth of *Colletotrichum capsici* at 300 ppm respectively. The second most valuable concentration is 200 ppm it reduced the 67.73% growth of *Colletotrichum capsici*. The 100 ppm concentration of Mancozeb showed least effectiveness in all concentration in reducing the colony growth of *Colletotrichum capsici.* It showed 60% growth inhibitions (Table 3) (Fig 2).

Table 2: Effect of different treatments of plant extracts on radial growth of *Colletotrichum capsici*

Treatments	5 (days)	10 (days)
Control	0.00 k	0.00 k
Eucalyptus 25%	2.10 d	2.53 c
Eucalyptus 50%	2.80 bc	3.00 b
Eucalyptus 75%	3.03 ab	3.17 a
Dodonaea 25%	1.07 gh	1.93 def
Dodonaea 50%	1.77 ef	2.57 c
Dodonaea 75%	2.03 de	2.83 bc
Garlic 25%	0.10 k	0.90 hi
Garlic 50%	0.47 j	1.23 g
Garlic 75%	0.73 ij	1.70 f
Means	1.41 B	1.9867 A

Means* within a column having same letters are statistically non-significant $p \leq 0.05$

Table 3: Effect of different treatments of Fungicide (Mancozeb) on radial growth of *Colletotrichum capsici*

Treatments	5 (days)	% Decrease over control	10 (days)	%Decrease over control	Means
Control	3.40cd		4.12a		3.76A
Mancozeb 10%	3.34c-e	01.76	3.90ab	5.10	3.62A
Mancozeb 20%	2.94ef	13.52	3.65bc	11.19	3.30B
Mancozeb 30%	2.67f	21.76	3.00d-f	27.00	2.83C
Means	3.09B	37.04	3.67A	43.29	

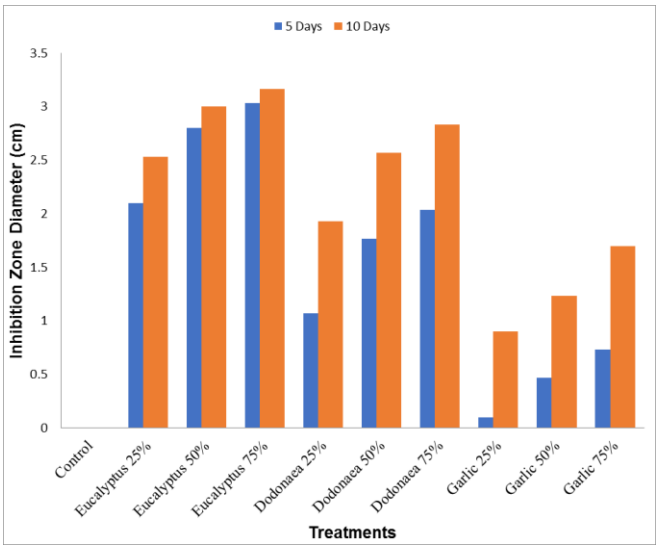

Figure 1: Effect of different treatments of plant extracts on radial growth of *Colletotrichum capsici*

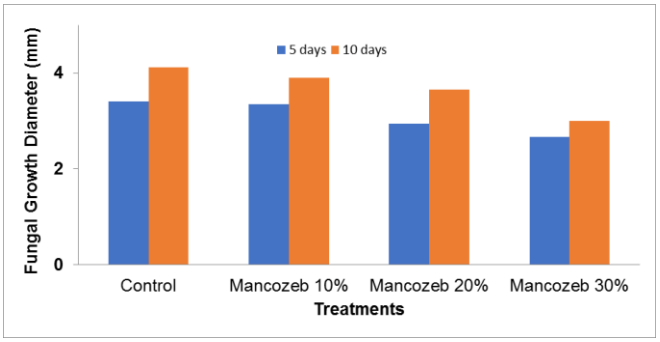

Figure 2: Effect of different treatments of Fungicide (Mancozeb) on radial growth of *Colletotrichum capsici*

***Conclusion*:** It was concluded that the extract of Eucalyptus was found to be the most effective and its inhibition zone was maximum. Dodonaea was having the second maximum inhibition zone and Garlic was third effective plant extract but the extract of Garlic was least effective against *Colletotrichum capsici*. On the other hand, Mancozeb showed the best result and it reduced the 71.7% growth of *Colletotrichum capsici* at 300 ppm. The second most valuable

concentration is 200ppm it reduced the 67.73% growth of *Colletotrichum capsici*.

REFFERENCES

Ajith, P. S. and N. Lakshmidevi. 2010. Effect of Volatile and Non-volatile compounds from *Trichoderma spp*. against *Colletotrichum capsici*incitant of Anthracnose on Bell peppers. Nature Sci.8(9).

Casilimas, H., O. Monsalve, C. R. Bojacá, R. Gil, E. Villagrán, L.A. Arias and L.S. Fuentes. 2012. Manual de producción de pimentón bajo invernadero. Fundación Universidad de Bogotá Jorge Tadeo Lozano, Bogota.

Harp, L. T., K. Pernezny, M. L. L. Ivey, S. A. Millar, P. J. Kuhn and L. Datnoff. 2008. The etiology of recent pepper anthracnose outbreaks in Florida. Crop Pro. 27:1380–1384.

Park, S., W. Y. Jeong, J. H. Lee, Y. H. Kim, S. W. Jeong, G. S. Kim, D. W. Bae, C. S. Lim, J. S. Jin, S. J. Lee and S. C. Shin. 2012. Determination of polyphenol levels variation in *Capsicum annuum* L. cv. c*helsea* (yellow bell pepper) infected by anthracnose (*Colletotrichum gloeosporioides*) using liquid chromatography-tandem mass spectrometry. Food Chem. 130:981-985.

Pereira, M. J. Z., J. N. S. Massola, A. A. B. Sussel, F. C. Sala, C. P. Costa and L. S. Boiteux. 2011. Reação de acessos de Capsicum e de progênies de cruzamentos interespecíficos a isolados de Colletotrichum acutatum. Horti. Brasil. 29: 569-576.

Than, P. P., R. Jeewon, K. D. Hyde, S. Pongsupasamit, O. Mongkolporn and P. W. J. Taylor. 2008a. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chili (*Capsicum* spp.) in Thailand. Plant Pathol. 57:562-572.

Mahasuk, P., N. Khumpeng, S. Wasee, P. W. J. Taylor and O. Mongkolporn. 2009. Inheritance of resistance to anthracnose (*Colletotrichum capsici*) at seedling and fruiting stages in chili pepper (*Capsicum* spp.). Plant Breed. 128:701-706.

Mongkolporn, O., P. Montri, T. Supakaew and P. W. J. Taylor. 2010. Differential reactions on mature green and ripe chili fruit infected by three *Colletotrichum* spp. Plant Disease. 94:306-310.

Aqil, F., M. Zahin, I. Alunad, M. Owais and M. Sajjad. 2010. Antifungal Activity of Medical Plant Extracts and Phytocompounds: A Review. *In*: Combating Fungal Infections: Problem and Remedy, Alunad, I., M. Owais and M. Shahid (Eds.). Springer-Verlag, Berlin Heidelberg. 449-485.

El-Badawy and Abdelgaleil. 2014. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Industrial crops and products. 52: 776-782.

Perez, C., M. Paul and P. Bazerque. 1990. An Antibiotic assay by the agar well diffusion method. Acta. Bio. Med. Exp. 15: 113-115.

Esimone, C. O., M. U. Adikwu and J. M. Okonta. 1998. Preliminary antimicrobial screening of the ethanolic extract from the lichen Usnea subfloridans (L). J. Pharm. Res. Dev. 3: 99-101.

Horburg, H. 1998. Influence of volatile extracts on storage pathogens of carrots *in vitro*. Vaxtskyddsnotise. 64: 87-89.