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EVALUATION OF DIFFERENT FUNGICIDES, BOTANICAL EXTRACTS AND BIO-CONTROL AGENTS AGAINST *ALTERNARIA ALTERNATA* THE CAUSAL AGENT OF LEAF SPOT IN SPINACH

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ABSTRACT

Alternaria disease is among the most important diseases of Spinach throughout the world. They affect primarily the leaves. *Alternaria* spp. is major plant pathogens, which cause at least 20% of agricultural spoilage most severe losses may reach up to 80%.This study was carried out to study the *in-vitro* efficacy of different fungicides, botanical extracts and bio-control agent against leaf spot disease of spinach caused by *Alternaria alternate.* Cabrio top showed minimum mycelial colony growth (23, 21 and 17 mm) at various concentrations 100, 200 and 300 ppm, respectively, followed by Topsin-M (44.5, 33 and 19 mm), Melodyduo (33, 26.5 and 22.5 mm), Prevail (43.5, 37 and 22.5 mm) and Antracol (41, 33 and 24 mm) at various concentrations 100, 200 and 300 ppm, respectively. However, in control, the mycelial colony growth of *A. alternata* causing leaf spot disease was recorded as 90 mm at 100, 200 and 300 ppm concentrations. Minimum mycelia colony growth (43, 35 and 15 mm) was recorded at 5, 10 and 15% concentration for Aak followed by Garlic (43.5, 33 and 20 mm), Neem (49, 38.5 and 23 mm), Onion (57.4, 50.25 and 27.65 mm) and Eucalyptus (57.9, 51.4 and 33.45 mm) and maximum mycelial colony growth (90.00 mm) were recorded in control. Minimum mycelial colony growth of *A. alternata* was observed for *Neurospora* spp. (47.00 mm) followed by *Hypocrea* sp. (48.00 mm), *Lasodiplodia* sp. (49.5 mm), Fusarium sp. (57.5 mm) and Chactomium sp. (57.5 mm). Maximum mycelial colony growth (90 mm) was recorded in control.

Keywords: *Alternaria alternate,* Biocontrol, Botanical extracts, Fungicides, leaf spot, Spinach

INTRODUCTION

Spinach (*Spinacia oleraceae L.*) locally known as Palak is an annual dioecious plant and belongs to Chenopodiaceous family. It is one of the most important winter leafy vegetable commercially grown in Pakistan. Hartmann *et al.* (1988). Spinach is an important vegetable of Rabi season and requires a cool and moist climate. Low temperature and high humidity help in the development of succulent, tenders mild flavoured foliage and quick growth. The plant prefers sunshine. Spinach is a rich source of fiber, vitamins A, C, E, K, B6, B2 and also magnesium, manganese, iron, calcium, potassium, copper, phosphorous, zinc, selenium, folate, betaine, folic acid, protein, niacin, omega-3 fatty acids, carotenoids beta-carotene and lutein and bioflavonoid quercetin with many other flavonoids. Spinach with poor source of fat is a suitable food for obese and diabetic people. It is also a good source of chlorophyll, which is known to aid in digestion (Bakkali *et al.* 2011; Verma, 2018). During the year 2017, the spinach production was stood at 109,403 thousand tons in Pakistan. The area under spinach cultivation was 8,763 thousand hacters (FAO, 2017). Downy mildew (Blue mold), bacterial soft rot, Fusarium wilt, Cucumber mosaic virus, Carpospores spot, White rust and Search Results Web results *Heterosporium* leaf spot can all be problems in spinach production (Batta, 2015). *Alternaria* spp. is mostly lead to leaf spot diseases in agriculture products. Spores (reproductive structure) of species of *Alternaria* are dim (dark brown to black) in colour and seem to look like in (felty black masses) on leaf and petal on plants surface. Spores are mostly transferred by the splashing of water or association of air. In several plants like zinnia, infection initiates in adulterated kernels. *Alternaria* infection can also spread from one source to another so management approaches must be an emphasis on all predisposed host plants (Chase, 1987; Bhale *et al.* 2015). *Alternaria* diseases are among the most common diseases of many grape plants throughout the world. They affect primarily the leaves, stems, flowers and fruits of annual plants, especially vegetables and ornamentals (Bharathi *et al.* 2014). *Alternaria* spp. is major plant pathogens, which cause at least 20% of agricultural spoilage; most severe losses may reach up to 80% of yield (Bhargava and Singh, 2012; Nowicki *et al*., 2012; Nizamani *et al*., 2020). *A. alternata* is commonly measured as an adjustable plant pathogen that expansions access into plant soft tissue through injuries or normal openings. In case of severe infection by the pathogen caused the infested leaf to turn brown and finally the leaf die with time (Cho and Moon, 1980; Boosalis and Hamilton, 2015).

MATERIALS AND METHODS

Survey and Sampling of infected Spinach: The samples were collected from Loralai. It is a municipality of central Balochistan. It lies between Punjab and Quetta valley. It is one of the ancient, cultural and historical places along the trade route from DG khan. It includes village killi Lahore, killi Pathankot, killi Zingwala, killi Shabozai and killi Dargi. It lies in between coordinates of 27º40'10"N and 85º21'18"E. It is situated at the altitude of 1325 meters and spread over the area of 11.47 sq. km. It has mild climate. It has an average rainfall of 20-37 cm. It is famous for the production of green leafy vegetables. Observations revealed that critical disease symptoms of leaf spot of spinach leaves were observed during the survey.

Isolation and identification of the causal fungus: The collected specimens were brought to Mycological laboratory at the Faculty of Crop Protection Department of Plant Pathology, Sindh Agriculture University Tandojam, for isolation and identification of the causal agent. The identification of fungus was done with the help of literature keys.

To evaluate the efficiency of different botanical extracts against the causal agents on the mycelia colony growth in vitro condition: Different botanical extracts like Neem, Akk, Garlic, Onion, and Eucalyptus etc. were checked against *A. alternata* causing leaf spot in Spinach. Which were evaluated against the causal pathogen by food poisoning method for this purpose 3 different concentration (5%, 10% and 15%) was incorporated in the PDA medium before the poring. Medium without fungicide served as control. After solidifying of the medium, 5 mm diameter agar disk of test fungus was cut from 8-10 days old culture plate by using sterile cork borer and placed in the center of the PDA plate. The inoculated plates were incubated at 25 ^{0}C. The radial colony growth of test fungus was recorded by drawing two perpendicular lines on the back of the petri plates crossed each other in the center of the plate. The data on colony growth was recorded along with these lines in millimeter after every 24 hours until the plates were filled in control. Followed by (Nizamani *et al*., 2020)

To test the effect of different biocontrol agents against *Alternaria alternata* under *in-vitro* conditions: Bio-control agents like *Trichodermaviride* and other biocontrol agents were evaluated against *A. alternata* the causal agent of leaf spot of Spinach. For this purpose, dual culture technique was used. Followed by (Nawaz et al., 2020)

To evaluate the efficacy of different fungicides: Furthermore, *in-vitro* studies of some selective chemical fungicides viz., Melodyduo, Cabriotop, Prevail, Topsin-M and Antracol fungicides were evaluated against the causal pathogen by food poisoning method. For this purpose, 3 different concentration (100, 200, 300 ppm) was incorporated in the PDA medium before the poring. Medium without fungicide served as control. After solidifying of the medium, 5 mm diameter agar disk of test fungus was cut from 8-10 days old culture plate by using sterile cork borer and placed in the center of the PDA plate. The inoculated plates were incubited at 25^{0}C. The radial colony growth of test fungus was recorded by drawing two perpendicular lines on the back of the petri plates crossed each other in the center of the plate. The data on colony growth was recorded along with these lines in millimeter after every 24 hours until the plates were filled in control.

RESULT

***In-vitro* antifungal properties of some fungicides at different dosage against the linear colony growth of *Alternaria alternate* causing leaf spot in spinach:** The results (Figure-1A,B,C,D,E and F) clarified that all the fungicides show a significant difference ($p<0.05$) in colony growth of *A. alternata* causing leaf spot disease in spinach. Among the fungicides, Melodyduo showed minimum mycelial colony growth (23, 21 and 17%) at various concentrations 100, 200 and 300 ppm, respectively, followed by Topsin-M (44.5, 33 and 19%), Cabriotop (33, 26.5 and 22.5%), Prevail (43.5, 37 and 22.5%) and Antracol (41, 33 and 24%) at various concentrations 100, 200 and 300 ppm, respectively. However, in control the mycelial colony growth of *A. alternata* causing leaf spot disease was recorded as 90% at 100, 200 and 300 ppm concentrations. Melodyduo ranked 1st, Topsin-M ranked 2nd, Cabriotop ranked 3rd, Prevail ranked 4th and Antracol ranked 5th for controlling colony growth of *A. alternata* causing leaf spot disease.

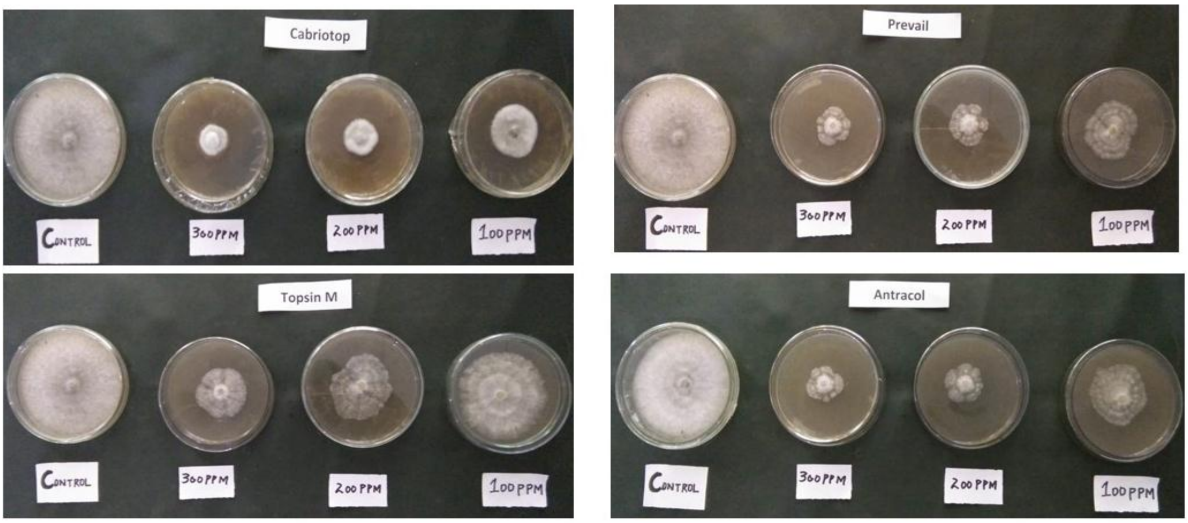

Figure 1 C, D, E & F. Effect of the different fungicides on mycelial colony growth of *Alternaria alternata* under *in-vitro* conditions.

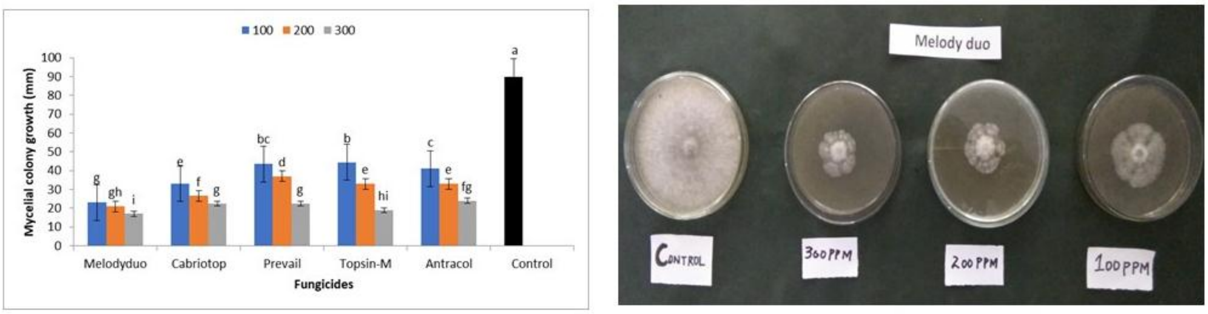

Figure 1 A & B. Effect of the different fungicides on mycelial colony growth *of Alternaria alternata* under *in-vitro* conditions.

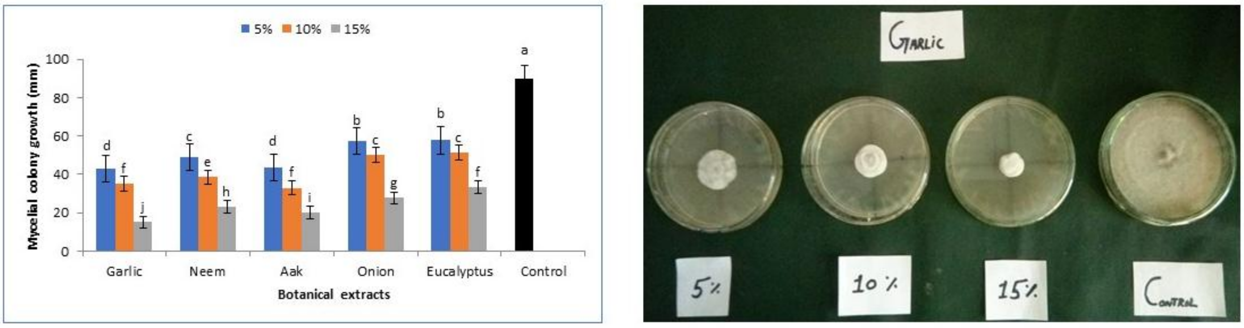

Figure 2 A & B. Effect of the different botancial extracts on mycelial colony growth of *Alternaria alternata* under *in-vitro* condtions

***In-vitro* antifungal properties of some botanical extracts at different dosage against the mycelial colony growth of *Alternaria alternata* causing leaf spot in spinach:** There was significant ($p< 0.05$) difference in colony growth of causative agent among the different botanical extracts and control. Results in regard to mycelial colony growth of *A. alternata* causing leaf spot in spinach through different botanical extracts are given in (Figure-2A, B, C, D, E and F). Numerically minimum mycelial colony growth (43, 35 and 15%) was recorded at 5, 10 and 15% concentration for Garlic followed by Aak (43.5, 33 and 20%), Neem (49, 38.5 and 23%), Onion (57.4, 50.25 and 27.65 %) and Eucalyptus (57.9, 51.4 and 33.45%) and maximum mycelial The results (Figure-15) indicates that minimum mycelial colony growth of *A. alternata* was observed for *Neurospora* spp. (47.00 %) followed by *Hypoxylon* Sp1 (48.00%), *Lasiodiplodia theobromae* (49.5%), Fusarium sp. (57.5 %) and Chactomium subaffine (57.5). Maximum mycelial colony growth (90%) was recorded in control. Statistically, there was significant ($p<0.05$) difference in mycelial colony growth between the biocontrol agents.lial colony growth (90.00%) was recorded in control.

***In-vitro* effects of the different biocontrol agents against *Alternaria alternate* causing leaf spot in spinach:** The results (Figure-3 A and B) indicates that minimum mycelial colony growth of *A. alternata* was observed for *Neurospora* spp. (47.00%) followed by *Hypoxylon* Sp1 (48.00%), *Lasiodiplodia theobromae* (49.5%), Fusarium sp. (57.5%) and Chactomium subaffine (57.5). Maximum mycelial colony growth (90%) was recorded in control. Statistically, there was significant ($p<0.05$) difference in mycelial colony growth between the biocontrol agents.

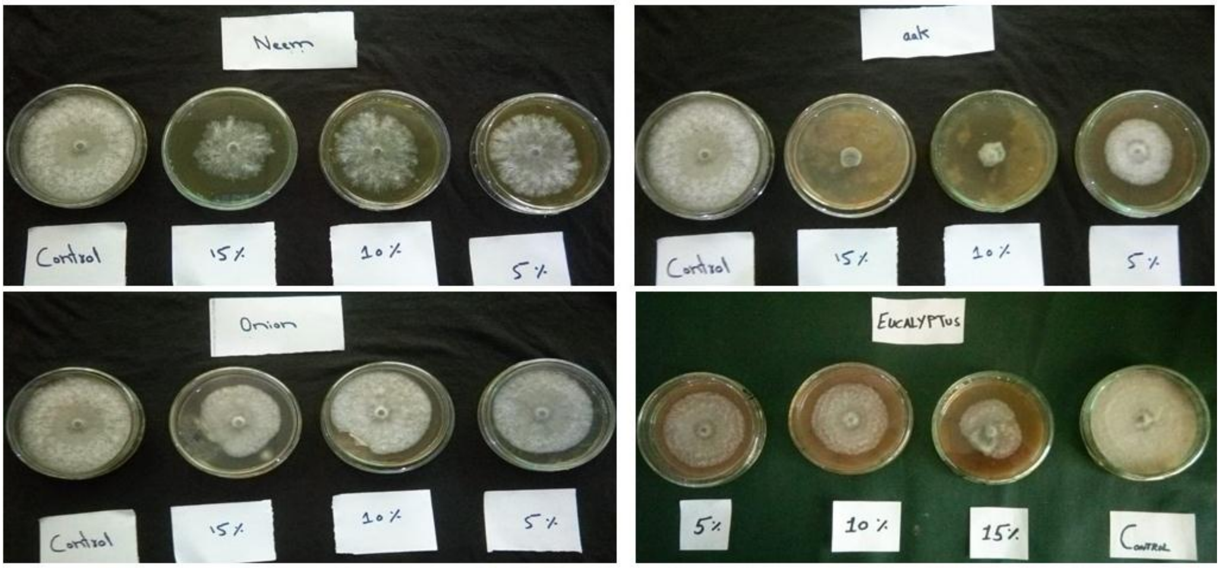

Figure 2 C, D, E & F. Effect of the different botancial extracts on mycelial colony growth of *Alternaria alternata* under *in-vitro* condtions

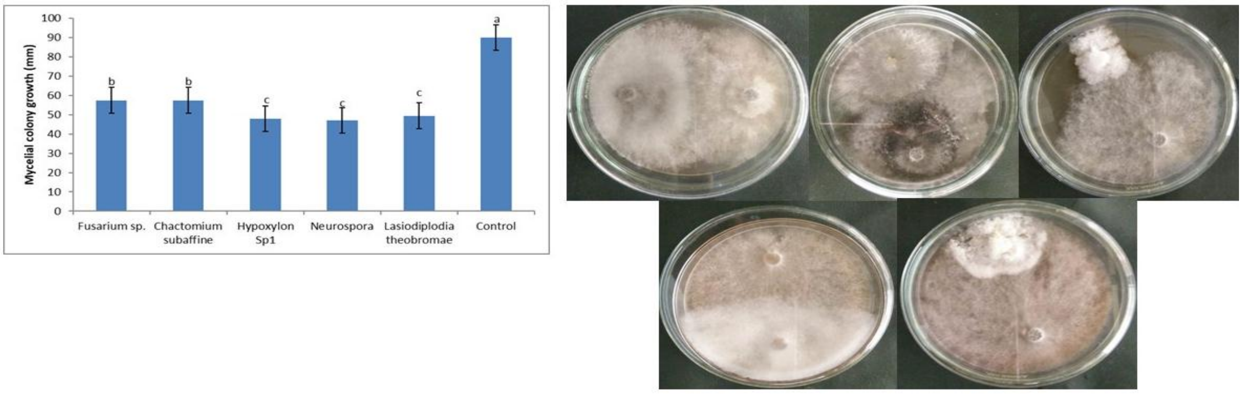

Figure 3 A & B. Effect of different biocontrol agents on the mycelial colony growth of *Alternaria alternata* under *in-vitro* condtions

DISCUSSIONS

Sample of diseased leave was collected and disinfected in the lab condition and on progress colonies, the pathogen was recognized via a light optical microscope. The most common underlying pathogen found was (*Alternaria alternate).* So, decontamination, duplication and conversation of *alternata* species were done for additional experimentation. It was concluded that chemical fungicide 'Melodyduo' was highly effective to control the mycelial colony growth of *Alternaria alternata* at 300 ppm. This finding of the current study is in agreement with the study of (Patil *et al.,* 2014) they stated that chemical fungicides reduced fungus growth significantly ($p<0.05$) better than botanicals; however, botanical also showed better response than control. The effectiveness of botanicals in post-harvest storage of marigold can be used at commercial scale. (Mesta *et al.* 2009; Gadhi *et al.*, 2020) by poisoned food techniques evaluated systemic and non-systemic fungicides against species of *A. alternata* of the systemic fungicides tested, highest average mycelial growth was recorded with Hexaconazole (94.44%), followed by Carbendazim (84.93%), Propiconazole (81.53%), Difenconazole (75.97%) and Thiophanate methyl (51.21 %). Of the non- systemic fungicides tested, Mancozeb recorded highest average mycelial growth (92.21%), followed by Curzet (84.45%), Chlorothalonil (80.90%), Propineb (78.89%) and Copper oxychloride (74.03%). Aqueous extracts of all the botanicals tested (@ 10 and 20%) were antifungal to the test pathogen. However, significantly highest average mycelial colony growth was recorded with *A. sativum* (74.45%), followed by *C. longa* (63.99%), *D.metal* (53.06%), *C. gigantica* (48.99%) and *P. hysterophorus* (48.90%). Sadana and Didwania, (2015) utilized seven fungicides to check fungi toxic viability against *Alternaria solani* viz captan, mancozeb, copper sulfate, thiram, carbendazim, Zineb and copper oxychloride. They utilized (500 ppm), (1000 ppm) and (1500 ppm) of every tested fungicide and found the most astounding decrease in infection by utilizing mancozeb at the amount of (1500 ppm) that brought on 85.04% limitation of mycelial development of *Alternaria spp.* Anwar *et al.* (2015) utilized miscellaneous fungicides (mancozeb trapeze artist and halonil) were measured against *Alternaria alternata* (Fr.) by using disc plate strategy against the causal agent of leaf spot infection.

Among the botanical extracts, Garlic extract showed better efficacy against mycelial colony growth of *Alternaria alternata* at 15% concentration. These results of the current study are in agreement with the study of Ashwani *et al.* (2016) stated that maximum antifungal potential was observed with the extracts of *C. sativa*, which recorded excellent inhibitory activity against *C. lunata* (100%), *A. zinnia* (59.68%), followed by leaf extract of *P. hysterophorus* (50%) against *A. solani*. Patil *et al*. (2001) indicated that the plant products, namely neem seed extract and neem leaf extract at 5% and tobacco decoction at 2% were effective in reducing disease incidence of tomato early blight caused by *Alternaria solani* and increased the fruit yield. Ambhore *et al*. (2003) reported that neem seed significantly reduced the intensity of leaf blight of irrigated wheat caused by *A. triticina*. Leaf extracts of neem and bishkatali were effective in controlling the leaf blight of cauliflower caused by *Alternaria brassicae* and *Alternaria brassicicola* (Hosna *et al*., 2003).

Among the biocontrol agent, *Neurospora* spp. showed better efficacy against mycelial colony growth of *Alternaria alternata*. These results of the current study are confirmed with the study of Gveroska and Ziberoski (2012) maximum figures in biological control of diseases literature discusses to the fungal genus known as (*Trichoderma*). Restricted use of chemical for active combined (IPM) management system (Monte, 2001). Mandare *et al*. (2008) signposted, the progress of pathogen *A. alternata* in research laboratorysituations is repressed by (*T. lingorum*), (*T. koningii*) and *T. virens*. Out of 16 weapons of biological isolates of species (*Trichoderma*) six destroy mycelialgrowth of more than 58% (Umamaheswari, Thakore, and More, 2009). These species arerecognized due to its capability to yield antibiotics and lithic enzymes (Lieckfeldt *et al*., 1999). Gang *et al*., (2004) stated that result is expressively better in rough than disinfected chitinase

CONCLUSION

On the basis of present findings, it was concluded that Melodyduo ranked 1st, Topsin-M ranked 2nd, Cabriotop ranked 3rd, Prevail ranked 4th and Antracol ranked 5th for controlling colony growth of *A. alternata* causing leaf spot disease. Among the botanical extracts, Garlic extract showed better performance in controlling colony growth of *A. alternata* causing leaf spot in spinach. Moreover, *Neurospora* spp. considerably retard the mycelial colony growth of *A. alternate.*

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