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# IDENTIFICATION AND MANAGEMENT OF *ALTERNARIAOCHROLEUCA*–A CAUSE OF LEAF NECROSIS IN MONEY PLANT

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## ABSTRACT

Diseases caused by fungal pathogens are very common to occur worldwide. Biological control is a method that offers harmless fungal management program and an alternative for dependence on chemical treatments. A survey was conducted in vicinity of Institute of Agricultural Sciences, University of the Punjab, Lahore and Money plant (*Epipremnum aureum*) was found to be infected with fungal leaf spot. Infected samples were collected for isolation, purification, and identification of the pathogen. The identification was carried out for morphological characterization and genetically from nucleotide sequencing of amplified ITS1-5.8S-ITS4 region of rDNA. *Alternaria ochroleuca* was identified as a leaf spot causing pathogen of money plant. Afterwards, pathogenicity aptitude of identified pathogen was confirmed by re-isolation of same pathogen from the artificially innoculated leaves of host plant using detached leaf method. Further to this, biological control of *A. ochroleuca* was carried out using methanolic extract of *Piper nigrum* L. (Black pepper) and *Amomum subulatum* Roxb (Cardamom). Both types of extracts presented varied results. However, all the employed concentrations of methanolic Cardamom extract suppressed the fungal growth except 1.5% concentration. Contrastingly, Black pepper extract did not inhibit the fungal biomass production. Therefore, Cardamom extract was considered to be more effective in controlling *A. ochroleuca* growth. Further studies will be carried out to fractionate different compounds from Cardamom and to determine the efficacy of these compounds against target pathogen.

**Key words:** *Alternaria ochroleuca, Amomum subulatum,* Biological control, Methanolic extract, Money plant, *Piper nigrum.*

## INTRODUCTION

Money plant is a perennial indoor plant of Family Araceae. The scientific name of money plant is *Epipremnum aureum*. It is a tropical vining plant found in many countries of the world including Northern Australia, Malaysia, Singapore, China, Japan, India and Pakistan. It is an attractive climber and known to have potential of removing internal air pollutants like formaldehyde, xylene and benzene (Sankar and Sree 2011).

Healthy money plant is resistant to most of the diseases and responds well to treatment when diseases are caught early. Fungi infect underground as well as above ground parts of the plants. Fungal leaf spot diseases are also one of the major problems for ornamental growers. Most commonly fungal pathogens involved in causing necrotic spots are *Alternaria, Phoma, Phomopsis, Edenia, Cercospora, Colletotrichum* (anthracnose) and *Myrothecium.* Spots caused by *Alternaria* species first appear as small necrotic lesions having water soaked appearance. Upon maturity these spots become darker in color sometimes with concentric rings and mostly sunken in appearance. Lesions are covered with black fuzzy growth (Laemmlen 2001) resulting in chlorosis and leaf fall (Pearce 2005) thus cause reduction in crop yields and economic losses (Montemurro 1992; Tsuge*et al*. 2013). *Alternaria* diseases are common on many ornamentals and cut flowers but they are not broadly explored.

A combination of cultural and chemical measures is used to control *Alternaria* leaf spot disease (Simone 1991). Pathogen free plants should be used upon a correct diagnosis. Fungicides that have been used in most of the cases toy control *Alternaria* diseases are mancozeb, iprodione, chlorothalonil, copper penta-hydrate and many copper compounds (Chase 1987).These composites regulate and eliminate infections and also create resistance in fungi. To diminish the progress of resistance by chemical over use, the high price of chemical fungicides as well as their lethal consequence, use of decomposable material like effective micro-organisms and fresh plant derived compounds of different parts gained significance in previous few eras for plant disease control (Duke *et al.* 2000; Bajwa *et al.* 2007). Natural plants derivatives subsidize a great in pathogens competition. Numerous families of plants such as Acanthaceae, Amranthceae, Apiaceae and Magnoliaceae possess antifungal and cytotoxic properties (Neerman 2003).Frequent studies in Pakistan also publicized a broader prospect of using extracts of plants for pathogens control (Bajwa *et al*. 2001; Ahmad and Abdelgaliel 2005). Plants derived spices are also used for the prevention of pathogenic and spoilage microorganisms. Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods.

Antimicrobial properties of certain spices have been reported in meat and meat products, e.g., poultry meat, turkey breast and beef, broth and foods (Nkanga and Uraih 1981). In current study, Black pepper and cardamom are used for biocontrol of *Alternaria* sp. that belong to family Piperaceae and Zingiberaceae, respectively. Biocontrol prospective of these plants is known worthy as they contain numerous natural substances that have antagonistic activities against microorganisms. Fungal leaf spot diseases are one of the major problems for ornamental growers. Pathogens on ornamental plants are not extensively researched, they are equally important like fruits and vegetables. The present study is therefore, designed to reduce hazards by ornamental fungal pathogens to minimum level.

## MATERIALS AND METHODS

**Sampling of Diseased Plant:** During a survey to study plants infected with fungal diseases to different localities of Lahore, Money plant (*Epipremnum aureum*) was found to be infected with necrotic leaf spots. The symptomatic leaves were photographed, collected in sterilized polythene bags and brought to laboratory for study. Data regarding the appearance, size, shape and color of the necrotic spots was recorded.

**Pathogen Isolation and Purification:** Isolation of fungal pathogen was carried out on Malt Extract Agar (MEA) medium as described by Dhingra and Sinclair (1993). For isolation of disease causing agent, 5 infected leaves and 2-3 necrotic spots per leaf were selected. Selected spots were cut into about 3 $mm^2$ pieces, surface sterilized in 1% sodium hypochlorite solution for 5 min. Eight to ten surface disinfectant leaf pieces were inoculated onto MEA under aseptic conditions and incubated at 25± 2˚C for 3-4 days. Hyphae from emerging fungal colonies were sub-cultured to fresh MEA petriplates and incubated at 25±2 ˚C for the purification of culture. Pure cultures were stored at 4 ˚C.

**Identification of Pathogen on the basis of Morphological Characterization:** Seven days old pure culture grown on MEA was used for morphology based identification. Complete phenotypic description of isolate based on macro and micro morphological characters was prepared. The colony characters observed were; color of culture and reverse, number of growth zones, colony diameter (cm), presence of aerial and submerged mycelia, type of conidial chains and abundance of conidia. Morphological examination was carried out according to Sime *et al.* (2002). Microscopic characteristics recorded were color, shape, number of conidia; number and position of conidial septa and conidial attachment with conidiophores, ornamentation of conidial walls, presence, size and shape of conidial beak and presence of apical pore(s). Species was key out by comparing its description with published authentic literature (Ellis 1971; Ellis 1976; Domsch *et al.* 1980; Simmon 2007). Photographs were taken for the record of macro - and micro - morphological characters.

**Identification of Pathogen on the basis of Genetic Characterization:** The internal transcribed coding regions of genome were amplified using the universal primer pair ITS1 (Forward) and ITS4 (reverse) using total fungal genomic DNA as template (Akhtar *et al.* 2014). Amplified PCR product was sent for nucleotide sequencing and resulting DNA sequence was analyzed by nucleotide BLAST. Identification of pathogen was confirmed based on maximum homology with the species sequences in the GenBank database. Nucleotide sequence was also deposited to GenBank.

**Pathogenicity Test:** To make spore suspension the protocol of French and Hebert (1982) was followed using 10 days old fungus culture. The culture plates were scratched with a sterilized spatula under aseptic conditions. Culture contents were added to saline tween 80 and vigorously shaken in a vortex mixture. Conidial number was adjusted to $3x10^4$ spores /ml with the help of a haemocytometer (Neubauer Precidor HBG, Germany).

**Detached Leaf Method:** This method was adopted to evaluate the pathogenic potential of isolated fungus. For this purpose, sterilized petriplates were floored with two filter papers in each petriplate moistened with 2ml double distilled water. The detached leaves of healthy plants were placed in petriplates in such a way that the petiole ends remained inserted in filter paper. Then 0.5ml of spore suspension ($3x10^4$) was inoculated on the leaf surface under aseptic conditions, incubated at 25+2 ˚C for 7 days and monitored regularly for disease appearance. Symptoms were observed after 1 week and the disease portion was re-inoculated onto media plates for the confirmation of the pathogenicity of isolated fungus.

**Pot Trial Method:** Pots were filled with soil at the rate of 350g/pot that was sterilized at 45˚C for 24 hours and seedlings of selected varieties were sown in pots. Then pathogenicity test was implemented by injecting spore suspension ($3x10^4$spores/ml) with a syringe on leaf surface and by spraying in soil. Control received same amount of distilled water. All plants were covered with polythene bags for 48 hours. Afterwards the plants were kept in shade under optimum temperature (25-26˚C) and watered when required.

**Disease Rating Scale:** Disease rating scale was constructed by observing disease incidence and disease severity. Disease incidence was witnessed as the symptoms appeared on the plant and disease severity was calculated using formula:

$$\text{Disease Severity} = \frac{\text{Area of plant part Affected}}{\text{Total Area}} \times 100$$

**Biological Control:** For biological control of *Alternaria* sp. Black pepper and Cardamom were selected. Fresh samples of spices were collected; grinded to fine powder and 50g of powder of each material was soaked in 100ml of methanol for 48 hours. Afterwards, materials were filtered and the filtrates were evaporated in hot air oven at 45°C to yield 2.42g and 5.84g of crude methanolic extract of Black pepper and Cardamom, respectively. Then methanolic extracts were liquefied in precise amount of sterilized distilled water to make stock solution of each plant separately.

Methanol residues were first dissolved in dimethoxysulfoxide (DMSO) and then sterilized distilled water was added to get the final concentration of 0.6g/ml. To check the bioactivity of extract, 5 concentrations viz., 0, 0.5, 1.0, 1.5, 2.0% of the organic solvent residue were formed. In 100ml flasks, 55ml malt extract broth was sterilized. Five concentrations 0,0.5, 1.0, 1.5, 2.0g/100ml were prepared by adding 0.5, 1.0, 1.5, 2.0 ml of stock solutions and 4.5, 4.0, 3.5, 3.0 ml sterilized distilled water respectively to each flask to make the total volume up to 60ml, while control received 5ml of distilled water and divided into four equal portions in 100ml flasks representing as replicates. A spore suspension (0.5ml) of *Alternaria* sp. containing $3x10^4$spores/ml was inoculated to all treatments and incubated at 28±2°C for 10 days.

The fungal biomass of all treatments was collected on pre weighed filter papers after 10 days and oven dried at 60 °C to get their dry weight yield (Bajwa *et al.* 2006). The growth response of fungus was calculated to assess the comparative effects of different concentrations of Black pepper and Cardamom methanolic extracts on fungus by following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

## RESULTS

**Study of Disease Symptoms:** Symptoms included irregular leaf spots with dark colored margins having the size ranging from 3-7 mm. Infected plants also showed yellowing around the necrotic lesions on leaves (Fig. 1).

**Identification and Characterization of Pathogen**

**Morphological Characterization:** Fungal pathogen was found to grow rapidly on malt extract agar medium (MEA) at 25 ±2°C reaching 4-5 cm in 7 days. Colony was black in color from both sides with velvety texture. Hyphae were brown and septate. Conidiophores were also brown, septate, and branched. Conidia appeared brown, muriform, ovoid or obclavate, with or without beak, also have elongated, beak-like apical cells, geniculate, often produced in chains and sometimes solitary, may have longitudinal as well as transverse septa, some have smooth and some have roughen walls. Conidial size ranged from 16-45 x 7-14 µm (Fig. 2). On the basis of morphological studies, pathogen was identified as *A. ochroleuca*. A vial of pure fungus culture was deposited to First Fungal Culture Bank of Pakistan (FCBP) under a unique accession number.

**Genetic Characterization:** Genetic characterization of species was carried out by sequence analysis of the ITS region by nucleotide BLAST analysis. A single compact band of fungal genomic DNA was detected on agarose gel. The consensus primers ITS1 forward and ITS4 reverse successfully amplified the ITS1–5.8S -ITS4 region of rDNA. A single band of PCR product of about 650 bp was observed on agarose gel (Fig. 3). The ITS nucleotide sequence when analyzed by BLAST using the National Center for Biotechnology Information (NCBI) website, showed 100% similarity with the GenBank *A. ochroleuca* strains DUCC5026 (KJ728681) (Fig. 4), DB-3 (FJ426387) and J8M-19 (JN226904).

**Confirmation of Pathogenicity:** Koch's pathogenicity postulates were applied to confirm the pathogenic potential of isolated fungus.

**Detached Leaf Method:** Initially detached leaf method was adopted to evaluate the pathogenic potential of isolated fungal culture (Fig. 5). A disease rating was developed according to infection and characteristic visible symptoms on inoculated leaves (Table 1). However control leaves remained asymptomatic. Disease symptoms started to develop within 2 days of inoculation when minute spots were exhibited on the surface of leaves in petriplates with approximately 10% infected area. After 2 to 10 days, a sharp disease progress curve was attained and 50% of leaf area was found to be infected (Fig. 6).The diseased portion of leaf from the petriplate was re-inoculated on media plates and re-isolation of similar pathogen confirmed the pathogenicity postulates.

**Pot Trials:** The results of pot trials conducted to determine the nature of infection and severity induced by pathogen on the host plant *in vivo* revealed that symptoms started to appear after 25 days of inoculation. Primarily plant leaves exhibited yellowing that was gradually turned into lesions showing chlorosis and necrosis, slowly the plant started to wilt. Severely infected plants showed spots on the whole leaves. Percentage infected area was recorded to calculate disease severity induced by *A. ochroleuca* (Table 2).

**Biological Control - Response of *A. ochroleuca* to Methanolic Extract of Black Pepper and Cardamom:** The *in vitro* antifungal potential of Black pepper and cardamom extracts was noted against *A. ochroleuca* and the findings are shown in Fig.7and Fig. 8. The results displayed a significant inhibitory activity of the extracts of cardamom. Methanolic extract of black pepper

exhibited encouraging results in stimulating the fungal growth than cardamom. However, variances in growth were apparent and erratic with respect to the employed concentrations. The results exhibited a significant decrease in fungal growth rate in every concentrations of cardamom however 0.5% concentration depicted the maximum arrest in fungal growth. A negligible decrease in biomass production was recorded in 2% concentration of cardamom extract, whereas in black pepper extract, a drastic increase in biomass was observed at the highest concentration (2%). However the percentage growth inhibition of *Alternaria* species in Methanolic extracts at 0.5, 1, 1.5 % concentration of Black pepper was 7.25, 0.9 and 6.75% respectively and of cardamom it was 3.25, 3.75 and 5.25%, respectively (Fig. 9).

**Fig. 1.Infected leaf of Money plant showing necrotic spots.**

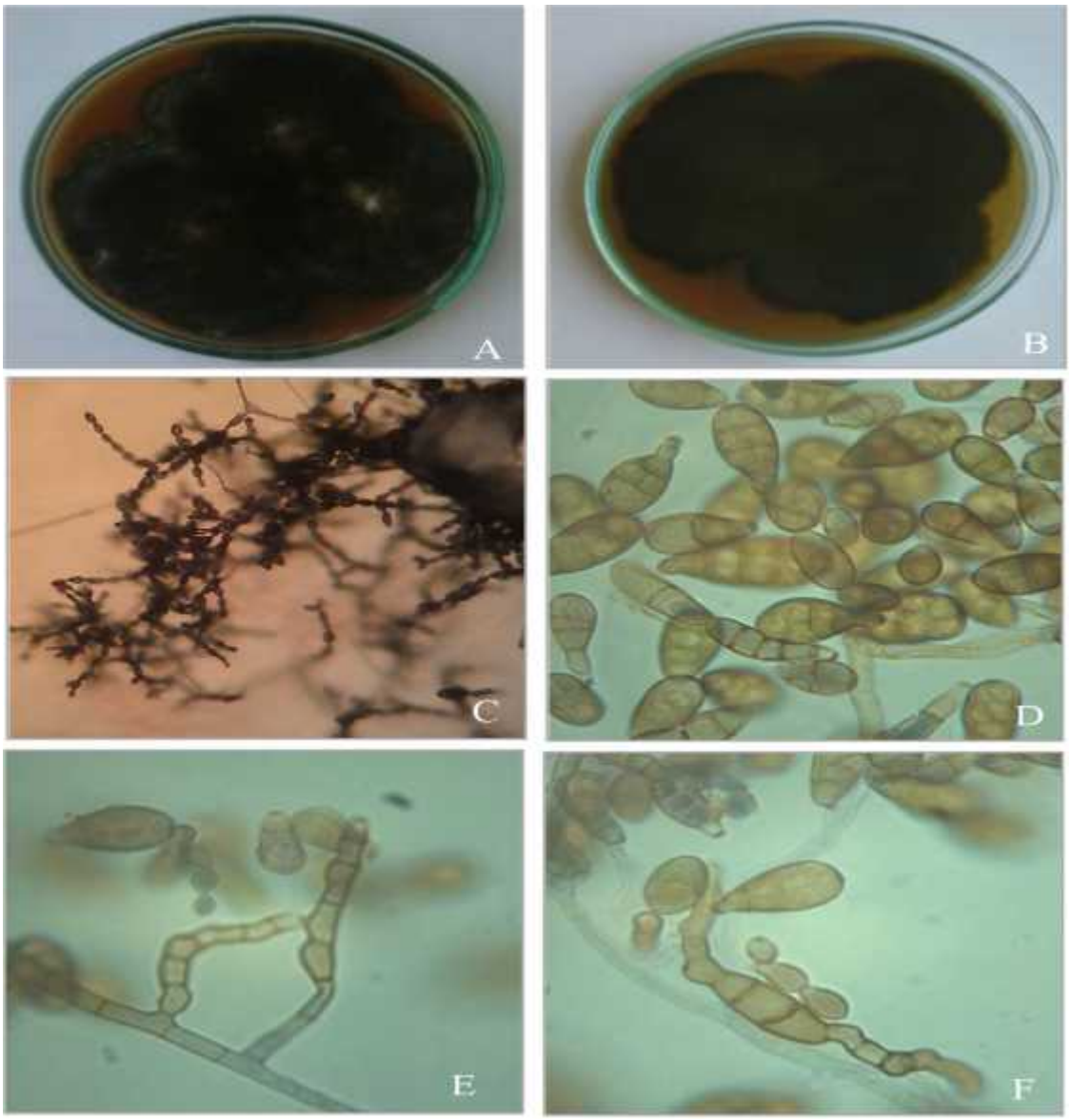


**Fig. 2. *Alternaria ochroleuca.* Colony (A) and reverse (B) on MEA; Mycelium and spore attachment (C) Conidia (D) Foot cell (E) and Conidia (F).**

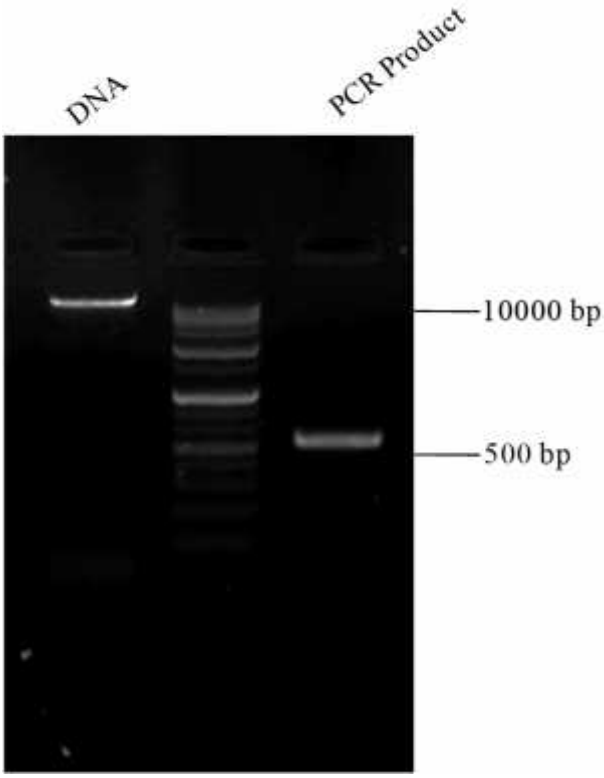


**Fig. 3.Agarose gel electrophoresis of total fungal genomic DNA and amplified ITS region of rDNA.**

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FCBP1529  1    GCGGGCTGGACCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTA  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  6    GCGGGCTGGACCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTA  65

FCBP1529  61   CTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTG  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  66   CTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTG  125

FCBP1529121  CAATCAGCGTCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTG180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  126  CAATCAGCGTCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTG  185

FCBP1529181  GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAAT240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  186  GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAAT  245

FCBP1529241  CATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAG300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  246  CATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAG  305

FCBP1529301  CGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGA360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  306  CGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGA  365

FCBP1529361  GACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCA420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  366  GACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCA  425

FCBP1529421  CTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCtttttttCAACTTTTGACCTCGGAT480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
DUCC5026  426  CTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACCTCGGAT  485

FCBP1529481  CAGGTAGGGATACCCGCTGAACTTAAGCATATCAA515
             ||||||||||||||||||||||||||||||||||
DUCC5026  486  CAGGTAGGGATACCCGCTGAACTTAAGCATATCAA  520
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**Fig. 4. ITS sequence alignment of *A. ochroleuca* with DUCC 5026 strain of *A. ochroleuca.***

**Table 1. Disease rating scale.**

| Infected area (%) | Score | Status |
|---|---|---|
| 01-20 | 1-2 | Highly resistant |
| 21-40 | 3-4 | Resistant |
| 41-60 | 5-6 | Moderately susceptible |
| 61-80 | 7-8 | Susceptible |
| 81-100 | 9-10 | Highly susceptible |

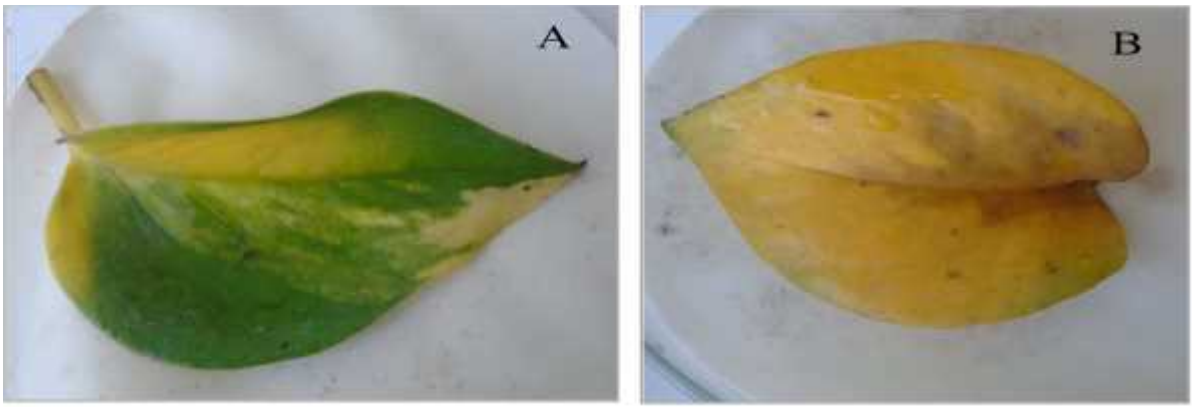


**Fig. 5. Progression of disease symptoms in inoculated leaves using detached leaf method. Start of disease signs (A) and fully infected leaf (B).**

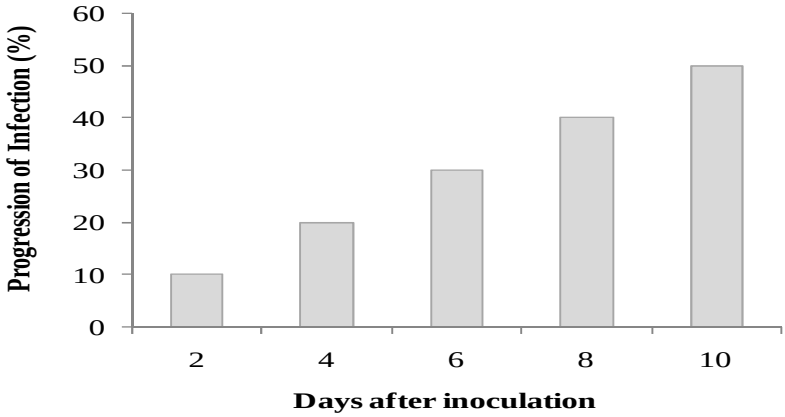


**Figure 6. Disease progress curve by detached leaf assay.**

**Table 2. Pictorial representation of disease rating scale on the basis of symptoms.**

| Key scale | Disease Description | | Disease severity |
|---|---|---|---|
| 0 | No symptoms |  | 0 |

| | | | |
|---|---|---|---|
| 1 | Yellowing started on the leaf |  | 10 |
| 2 | Wilting with spots |  | 30 |
| 3 | Spots increase on leaf and reduce growth |  | 50 |
| 4 | Whole plant is infected with spots |  | 70 |
| 5 | Complete death of plant |  | 100 |

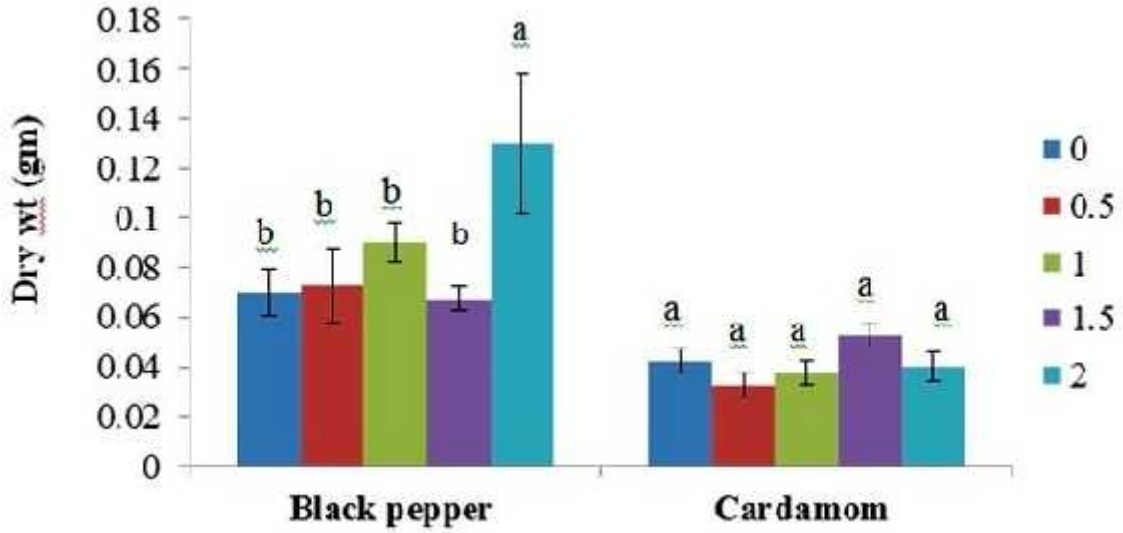


**Fig.7. Effect of different concentrations of Methanolic extracts of Black pepper and Cardamom on the biomass of *A. ochroleuca.***

Vertical bars indicate standard error of means of four replicates. Values with different letters show significant difference as determined by Duncan's multiple range (DMR) test

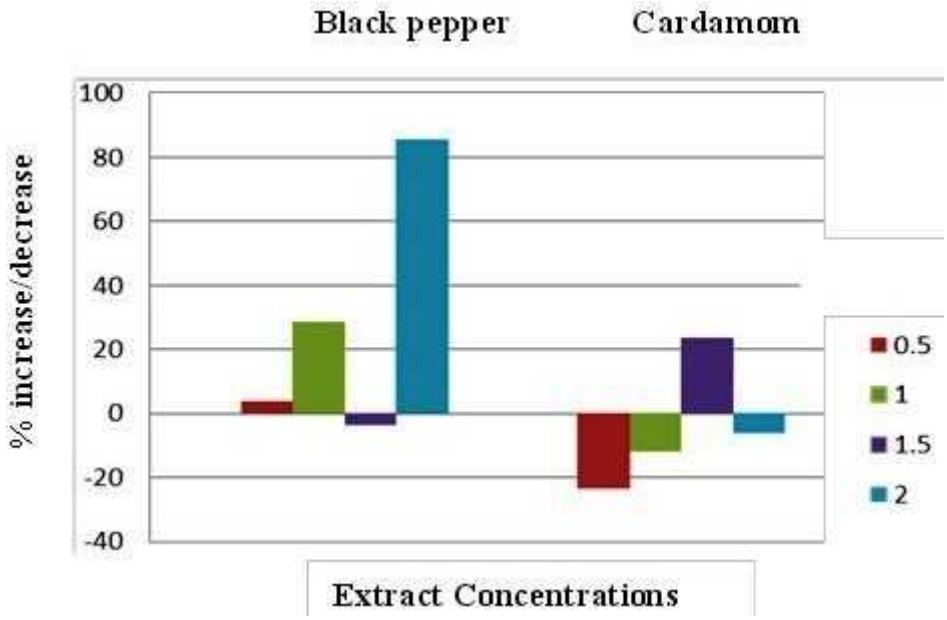


**Fig.8. Effect of different concentrations of Methanolic extracts of Black pepper and Cardamom on % increase/decrease of *A. ochroleuca.***

## DISCUSSION

*Alternaria* species are common field fungi, including saprophytic and pathogenic species causing infections in cereals, vegetables and fruit crops (Bottalico and Logrieco 1998). *Alternaria* taxonomy is mainly constructed on morphology and development of conidia and conidiophores. Indeed, morphology is still considered as the most reliable method to identify *Alternaria* at the species level, but misidentifications are known to occur (Anderson *et al.* 2006). Consequently, a number of molecular approaches have been established to assist differentiation among species i.e., analysis of ribosomal DNA (rDNA) sequences to find molecular phylogenetic relationships within many groups of fungi (White *et al.* 1990; Mirhendi *et al.* 2007) or by using the mitochondrial small subunit (SSU) rDNA sequence method (Kretzer *et al.* 1996). Presently, *A. ochroleuca* was identified as a leaf spot causing pathogen of money plant microscopically for morphological characterization as

well as genetically from nucleotide sequencing of amplified ITS1-5.8S-ITS4 region of rDNA. Akhtar and colleagues (2014) in their study isolated and identified *Alternaria* sp from the different infected tissues of diseased canola plant on morphological basis. In the contemporary lines Bashir *et al.* (2014) isolated and identified *A. metachromatica* on the basis of complete description of macro and microscopic characters followed by identification using rDNA spacer sequence and revealed that *A. metachromatica* was the causal agent of leaf spot of tomato.

In the current research the phytotoxic potential of *A. ochroleuca* was confirmed by applying Koch's postulate using detached leaf method and pot trials. The results portrayed that *Alternaria* spp. had the effectiveness to encourage leaf spot on plants. Infection and characteristic visible symptoms were evident on inoculated leaves and whole plants exhibiting a sharp progressive disease curve with 50% of infected area. *Alternaria* spp. induced the extreme symptoms as dark brown lesions on leaves which ultimately led to plants death within few days of inoculation. Infection caused severe defoliation. Earlier in India, *A. alternata* has been reported as a cause of leaf spot on Money plant. Working in the parallel lines Shafique *et al.* (2013) reported the use of detached leaf assay to confirm the pathogenic potency of isolated pathogen. Earlier Mahmood (2010) also reported the similar drift of disease progression in tomato by *A. alternata*.

Thus, *A. ochroleuca*is identified as a plant pathogenic fungus inducing substantial losses innumerous plant species which must be controlled. Spying of fungitoxic potential of natural plant derived compounds for managing fungal pathogenesis becoming an important part of research these days. Abundant studies accompanied in Pakistan publicized a largescale prospects of manipulating plants extracts for management of fungal pathogens (Bajwa *et al*. 2001; Ahmad and Abdelgaliel 2005; Braga *et al*. 2007). By understanding the significance of biological control system to control pathogen, current study was intended to testify fungitoxic potential of Black pepper and Cardamom against phythopathogenic fungus *A. ochroleuca*. Despite of its wide spectrum use as a food additive, The Black pepper is also used as a drug in Indian and Chinese systems of medicine (Atal *et al.* 1975; Nadkarni 1976; Kurup *et al.* 1979). Volatile oil, an active constituent of spices, impart antiseptic, antibacterial, antifungal properties (Subramanyam *et al.* 1957). Antifungal properties of essential oil of Cardamom were demonstrated against *Aspergillus niger*, *Geotrichum* sp. and *Rhodotorula* sp. as well as *Fusarium oxysporum*. The active plant metabolites may play a significant role in disease control, identify effective active compounds of plants, moreover recognize solvent and appropriate methods of plant metabolites extraction, to find non-chemical compounds against plant diseases. Piperine is the major constituent of oil that can be extracted from black pepper while 1, 8-cineole is the major compound found in small Cardamoms. One or more of these compounds may be responsible for mycotoxic activity of these extracts against the pathogen. Plants with secondary metabolites can be used in form of powders, extracts and essential oils against plant disease (Behdani *et al*. 2012).

In current research work, methanolic extracts also displayed substantial fungitoxic activity. The greatest antifungal stress was brought by 2% concentration of black pepper and cardamom extract triggering depression of about 50% and 80%.respectively, in dry biomass production of *A. ochroleuca*. These conclusions are also in accord with the effort of Fardos (2009) who stated that among 5 different plants tested, lemon grass was the most effective against different pathogenic fungi including *Microsporum canis, M. gypseum* and *T. mentagrophytes.* Bobbarala *et al.* (2009) also suggested that methanolic extracts of plants are helpful in treating diseases of plants. Presently methanolic extract of Black pepper exhibited promising results in stimulating the fungal growth at higher concentration as at 2% concentration, there was more than two fold increase in fungal growth. The enhancement of biomass production of *Dreschlera hawaiiensis* at higher concentration of shoot extracts may be attributed to detoxifying ability of the fungi, to allelochemicals (Sicker 1998). It may be due to the ability of some allelochemicals to enhance the growth of mycoflora (Mughal *et al*. 1996) or ability of particular species to exploit them as source of nutrition. The current work infers that methanolic extracts of Black pepper and Cardamom retain potent antifungal combinations against *A. ochroleuca*, which may grasp robust antifungal activity and can be exploited as an ultimate strategy for plant disease management programs to eliminate fungi.

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