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Antifungal Potential of Selected Medicinal Plants Against Post-Harvest Fungi of Tomato

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ABSTRACT

Tomato belongs to the Solanaceae family. It is an essential vegetable crop of Pakistan. Production of tomato affected by viral, fungal, and bacterial diseases. Some post-harvest tomato fungal pathogens like *Aspergillus*, *Alternaria*, *Fusarium* and *Rhizopus* are major contributors to tomato fruit losses. The current research was focused to control these post-harvest fungal problems of tomato by application of some botanical extracts because these have no harmful residual effects like fungicides which influence fruit value and human health. For this purpose, infected samples were collected from a local market. Then fungi were isolated from these infected samples on PDA culture media. These purified fungal cultures were inoculated to healthy tomato fruits to confirm the pathogenicity of these fungal isolates. During management trials inoculated fruits from each group were coated with different concentrations of *Curcuma longa*, *Mentha piperita* and *Ocimum basilicum*. A completely randomized design (CRD) was followed in these experiments with three replications. Data regarding the infected fruit area was recorded following the standard procedures Fisher's analysis of variance technique was used for analyzing the data and a significant differences test (LSD) was used for comparing differences among treatments using least at 5% probability. The turmeric plant was more efficient than Basil and mint. At 200ppm concentration, the turmeric inhibits 80.1 % growth of *Alternaria alternata* on PDA media, but the growth rate was reduced up to 31.6 % at 50 ppm concentration. Growth inhibition significantly reduced as concentration increased from 50ppm to 200 ppm concentration.

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INTRODUCTION

Agriculture is the backbone of Pakistan's economy, where most growers depend on agriculture for support. More than 70.6 per- cent of people belong to rural areas and most of them are connected to agriculture and livestock. In the recent past, the agricultural and industrial sectors

have faced many ups and downs, and all agricultural growth has remained contagious (Altaf, 2008; Adil *et al.*, 2004). Agriculture is the backbone of the economy in developing countries and is essential to improve the rural population's livelihood through improved technology (Cambell and Barker, 1997).

Tomato (*Lycopersicon esculantum* L.) initiated from South America belong to the family Solanaceae. It is broadly grown vegetable in all over the world with a height of at least 1.3m (Acquah, 2002). The tomato plant is about 100-300 cm tall and has a slightly woody stem and a bit thin. It has bushy appearance and at maturity decides the form of bear fruit. It is prominent and essential vegetable crop of Pakistan. Its production and supply are limited and almost fluctuating throughout the year from early summer to mid-winter (Hussain *et al.*, 2001).

Tomato production and the cultivated area is growing from an output of 354,356 tonnes in 2009 and to the 407,374 tonnes of cultivated area in 2011 from 17,230 ha to 18,178 ha worldwide. It is grown over a large 4-million-hectare land (FAO, 2003). Tomato in Pakistan grow approximately 61,000 hectares with a production of 572.8 thousand tones and an average yield of approximately 9.4 tones/ha. (GOP, 2018). Sindh's share of tomato fruit in 2015 is 35.62 percent, Khyber Pakhtunkhwa (kpk) by 22.92 percent, Baluchistan 24.86 percent and Punjab 16.57 percent (FAO, 2015). Tomato was manufactured at 413610 MT and 359935 MT in Bangladesh throughout 2013-14 and 2014-15, with approximately 5428 MT and 5188 MT coming from the district of Jessore, 70201 MT and 54805 MT coming from the district of Dinajpur, 4490 MT and 6800 MT coming from the district of Mymensingh and 3540 MT and 6241 MT coming from the region of Kishoregonj in 2013-14 and 2014-15 respectively.

Tomato is a rich source of lycopene which gives red colour during tomato ripening. Lycopene is an important natural antioxidant that is used in medicines. While tomato is a potential vegetable, it can be used as a cooked and possessed fresh commodity in various foods in Pakistan, including mashed potatoes, juice, tomato sauce and ketchup. China has the highest production of tomatoes compared to the United States, which is second. Turkey, Egypt, Italy, and India are the other big growers with a total of 5.6 million tones. Per capita the largest user in America, Canada, and Italy. China, Brazil, Spain, Greece, and Turkey are the major producers of 1, 1 million tonnes per annum of tomato production. Tomato production is 153 million tons per annum in the world (FAO, 2009).

Production of tomatoes can assist as an income source for the most rural makers in all the world's established countries (Arah *et al.*, 2015). Production of tomatoes is influenced by both biotic and abiotic factors. Biotic

considerations include bacteria, nematode, fungi, and viruses (Hammond & bionco, 1992). Fungal diseases are the most effective and widespread pathogens, causing a wide variety of congregation plants and creating damaging and thriftily significant losses during storage and transport of most fresh fruits and vegetables (Sommer, 1985).

The development of tomatoes worldwide is heavily affected by various diseases varies from infectious, fungal, nematode, and bacteria. *Alternaria alternata* causes Alternaria stem canker. The following are tomato fungal diseases. *Collectrichum corcodes* and *C. dematium*, *C. gloesporoides*, *Pleospora tarda*, *Stemphyllum botryosum*, *Thielavopsis basicola* black rot, *P. herbarium*, *Chelara elegaus*, *P. nicotiana*, *Phytophthora capsici*, *P. dreshleri* and A-caused black foot. Alternaria: Alternaria.

The fungus also affects cell wall, organic acid, and enzyme degradation (El-shaieb and Malibari, 1995; Hirsch *et al.*, 1995). Also, in developing countries, the pathogen declines harvested fruit at about 20-25 per cent during post-harvest handling (Droby, 2005; Zhu, 2006). Different species of pathogens such as *Mucor piriformis*, Alternaria and *Penicillium expansum* degrade the class of the fruits, minimize the market value, and these are not suitable for a human environment. Post-harvest fungal rots are regulated by the use of many chemical products but in the presence of the new pathogen breeds, the use of chemical develops resistance to fungicides and also harmful to human health and the environment (Spotts and cervantes, 1986).

Tomatoes with post-harvest losses can reach up to 25-42 per cent all over the world (Rehman *et al.*, 2007). These are all losses that result in poor recital for producers, workers, and all traders as well as for foreign income sufferers throughout the country (Kader, 1992). More tomato losses can be either measurable or qualitative. Even though today 's importance on crop research varies more from quantity to product quality (Oko-Ibom *et al.*, 2007), there is a slight improvement in the tomato species that are commercially grown for different purposes which are caused by the heavy losses.

Fungicides are commonly used to control post-harvest disease-causing pathogens, but these chemical agents harm human health and all other living organisms. Moreover, during the storage period due to the short time interval among analysis and utilization of horticultural products the use of this fungicide become more deleterious. Due to excessive use of a large number of

chemical agents such as benzimidazoles, imazalil and prochloraz some fungal pathogens become resistant and some of them like Rhizopus and Mucor require distinctive fungicides for their control (Nakahara *et al.*, 2013).

For the safety of human health and environment, plant products from various ecosystems have been collected as natural products and examined to check their chemical characteristics, medicinal importance, (Ghosh-Hazra and Chatterjee, 2008) ability to remove harmful oxidizing agents, (Lim and Murtijaya, 2007) and their ability to preserve food and to control pathogens (Perez-Pacheco *et al.*, 2004). Plant extracts from different plants have been examined to check their activities to control fungal pathogens (Daferere *et al.*, 2003), and to remove harmful oxidizing agents (Skerget *et al.*, 2004).

Plant extract is used as an alternative to conventional antifungal chemicals, as it is less toxic and more suitable than synthetic compound (Jobling, 2000). Plant extract that, due to its antifungal and antibacterial activity, gained prominence and scientific interest (Lee *et al.*, 2007; Verastegui *et al.*, 2008; Santas *et al.*, 2010). In established countries more than 800 million people do not have sufficient food resources and food is at least 10 per-cent loss due to all plant diseases (Strange and Scott, 2005).

Medicinal plant usage the 25,000 higher plants for photoactivity control in which 2,400 plant species were screened (Khafagi and Dewedar., 2000). The plant-based pesticide is available locally, is easily biodegradable, is inexpensive, and is not harmful. Early evidence suggests that many species of plants possess antibacterial and antifungal properties (Nduagu *et al.*, 2008; Yasmin *et al.*, 2008). Plants produce secondary metabolites, in which antifungal activity is mainly present. Plants include compounds such as phenolic, flavonoids, Sulfur compounds, phenolic glycosides, saponins, glycosylates, unsaturated lactose, and cyanogenic glycosides (Bennetl and Wallsgroove., 1994; Osbourne., 1996).

Basil plant (*Ocimum basilicum*) belongs to the family Labiatae. It is native to Europe, India, Turkey, Africa, and Asia (Amin, 2005). Basil is also known king of the herb due to its aroma. In medicine basil is used for the cure of asthma, tenderness of respiratory, stomachic, caught, antispasmodic, and urinary tract (Simon *et al.*, 1999). In the agricultural and medicinal field reportedly extract of basil has been used to inhibit the fungal pathogen growth such as Staphylococcus sp., Fusarium sp., Enterococcus sp., Aspergillus sp., listeria sp., and E.coil (kocic-Tanackov, *et al.*, 2011).

Turmeric (*Curcuma longa*) belongs to the family Zingiberaceae, is an evergreen herbaceous plant and commonly cultivated in Asia and mostly in India and China (Kapoor, 2000). It is the most important spice in Bangladesh used as a holy spice for their religion that having substantial quantities of proteins (6.3%), carbohydrates (69.4%), lipids (5.1%) and fiber (2.6%). Turmeric is a rich source of minerals like calcium phosphorus, vitamin A and iron (Hossain *et al.*, 2005).

Curcumin mostly does not dissolve voluntarily in water, although it is resolvable in all the organic solvents including ethanol, methanol, dimethyl sulfoxide, or acetone, and having a melting point of 183 ° C. The turmeric compound contains proteins (6.3%), carbohydrates (69.4%), moisture (13.1%), fats (5.1%) and minerals (3.5%) (Prasad *et al.*, 2014). Using nuclear factor-kB hang-up and cytokines, curcumin can protect the skin by foraging free radicals and reducing inflammation (Thangapazham, *et al.* 2007). Inflammatory cytokines causing allergy and asthma that are inflammatory disorders are harmful to human health (Shehzad, *et al.*, 2013). Turmeric tubers used in asthma and allergy treatment exclusively in India and for the treatment of burning and other skin diseases in Thailand (Tewtrakul and Subhadhirasakul, 2007).

This study will be focused to compare the efficacy of distinct desert plants part like *Curcuma longa*, *Mentha piperita* and *Ocimum basilicum* growing in Pakistan and examine the effect of the main fungal pathogens *Alternaria alternata* on some economically important fruits especially strawberry.

The objectives of the studies were Isolation, Identification, and purification of fungus from infected fruit samples and the assessment of the different plants extracts as fruit coating materials against *Alternaria alternata*.

METHODS AND MATERIAL

Collection of samples

The infected samples of tomato fruit were collected from different markets and storage houses located at Faisalabad in a polythene zipper bag and labeled properly with a permanent marker and were taken to the laboratory of the department of plant pathology.

Purification and isolation of fungus

The fungus was observed and isolated from different infected tomato samples. About 2-3cm rotted portion of infected tomato samples was separated and cut into small

pieces. Then the surface was sterilized with 0.1% NaCl and afterward treated sample rinsed two times with sterilized water and dried the sample with sterilized filter paper. Small pieces of diseased part were transferred on to the surface of PDA plates. For removing and inserting disease parts of infected samples needles, scalpels, forceps, and scissors were sterilized by dipping in the methylated spirit burning many times. The inoculated Petri plates were sealed with paraffin and incubate at $27\pm 2^{\circ}\text{C}$ for 36 hours. Within 24-36 hours, a fungal colony appeared on potato dextrose agar (PDA) media. Several fungi were isolated, identified, and purified through different fungal growth patterns, types of sporulation, and colony color. The specimens were identified with the help of literature (Booth, 1971, Sutton, 1980).

Artificial inoculation

For artificial inoculation, the healthy tomato was damaged by using a sterilized needle. Maximum 20-30 holes were made and marked the injured hole by a circle. With the help of a cotton swab the fungus were placed on these holes and the samples were covered with polythene bags at room temperature. After 10 days, rotting symptoms were observed.

Collection of plant extracts

The desert plants Mint (*Mantha piperita*), Basil (*Ocimum bacilicum*), and Turmeric (*Curcuma longa*) were collected from the experimental field of the University of Agriculture; Faisalabad (Pakistan) and brought to the laboratory of the department of plant pathology for further usage. For the process of preparation of extract plant parts cut into small pieces separately, then ground in the blender (food processor). Different plant parts are used to make extracts like leaves, roots, and stems, etc. First of all, the prepared plants extract was pass through an uncontaminated muslin cloth and then pass-through filter paper. (Ilyas, *et al.*, 1997). Different concentration of plants extract was used against *Alternaria alternata*.

Management through plants extract

Different medicinal plant extract like Mint (*Mantha piperita*), Basil (*Ocimum bacilicum*), and Turmeric (*Curcuma longa*) were used against the fungus that causing post-harvest losses. Ethanol was used to prepare 50ppm, 100ppm and 200ppm concentration of plant extract.

Processing of plant material

The collected plant materials (bark, wood and fruits) were surface sterilized with 0.1% HgCl_2 and washed three times with the sterile distilled water. Properly cleaned plant materials were dried in shade. Air-dried

plant materials cuttings were powdered using a powdering mill to 50 mesh size. Seeds were separated by depumping the fruits and powdered after proper cleaning and drying. Powdered plant materials were stored in sterile cellophane bags in a cool dry place till further use.

Extraction of plant material

The ground plant materials i.e. bark, wood and fruits (300gm each) were extracted with methanol in a Soxhlet extractor for 8 to 10 hours. The process was run till the decolorization of the solvent, after that the extract was sieved with Whitman filter paper (No.1) and the filtrates were concentrated using a rotary evaporator. Then the extracts were evaporated to dryness over a water bath and solvent-free extracts of respective parts were obtained (Nostro, *et al.*, 2000). Extracts of wood, bark and seeds of plant materials were weighed and then kept in labelled sterile sampling bottles.

Coating

Take fresh tomato fruit that was injured artificially by using a sterilized needle and labeled with a marker. In Petri plates put fungus with the help of cotton swab. Cover the complete fruit surface by coating it with plant extract with different concentration i.e.10%, 20% and 30%. In polythene bags put all the material and after 3, 6- and 9-days interval rotting symptoms will be checked.

Statistical analysis

In vitro disease management through medicinal plant extract was approved into the laboratory condition under completely randomized design (CRD). Means of all experiments were compared by using the least significance difference (LSD) at 5% prospected value. (Steel *et al.*, 1997).

RESULTS

Effect of medicinal plant extract on the radial growth of *Alternaria alternata* on potato-dextrose agar

Statistical data were shown in Figure 1. Three different concentration were made like 50, 100 and 200ppm of *Curcuma longa*, *Mentha piperita* and *Ocimum basilicum* plant extract. Fungal growth was inhibited continuously with the increasing concentration of medicinal desert plant extract from 50ppm to 200ppm. Maximum inhibition of *Alternaria alternata* was observed at 200ppm concentration of *Curcuma longa*, (67.5%), *Mentha piperita* (65.33%) and *Ocimum basilicum* (58.85%) after 3 days and 81.5%, 75.33% and 70.83% after 7 days respectively. Different concentrations were applied to different replications

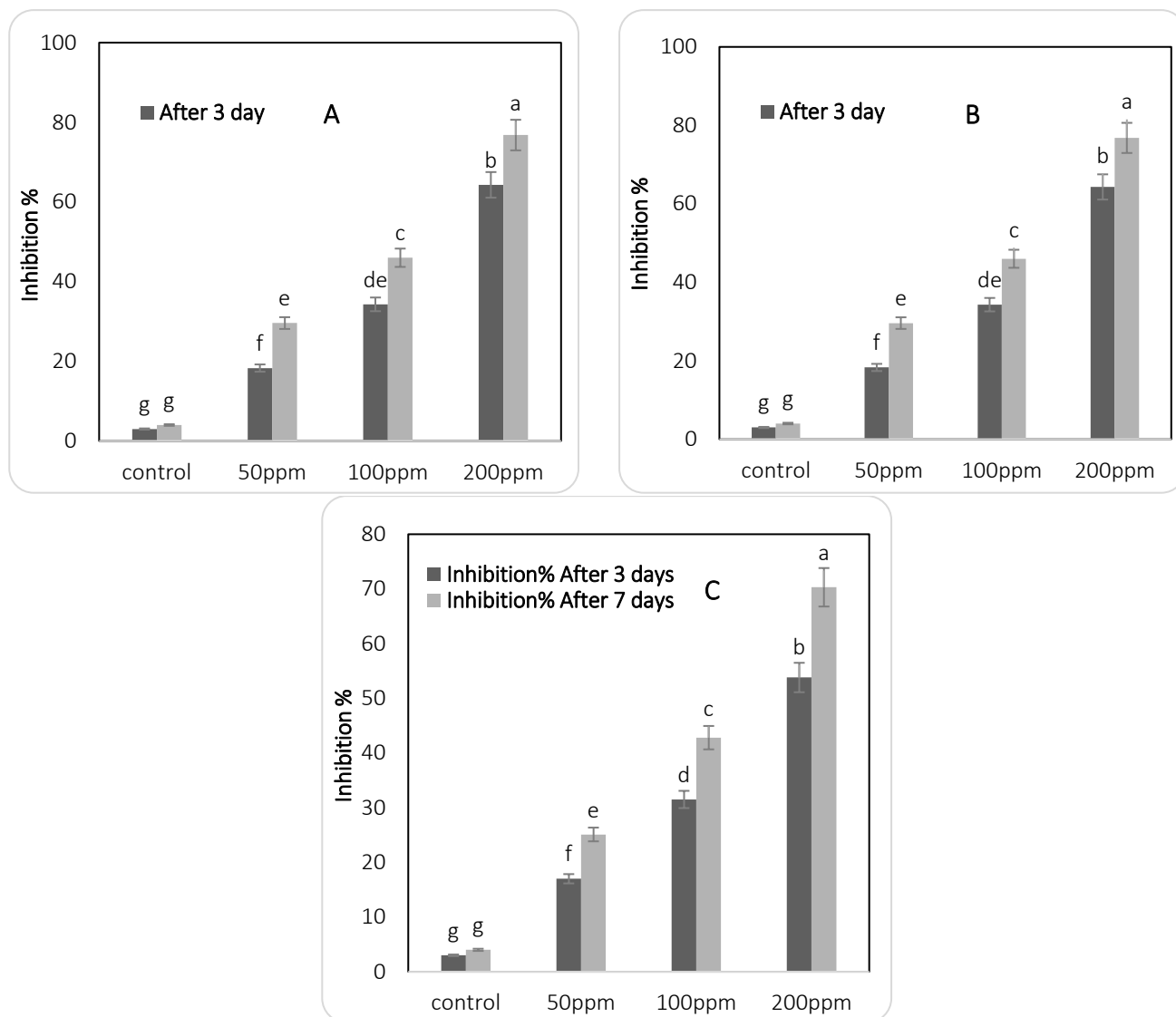


Figure 1. Effect of medicinal plant extract of *Curcuma longa* (A), *Mentha piperita* (B) and *Ocimum basilicum* (C) on the radial growth of *Alternaria alternata*. This graph shows the average values of the three replications. LSD test was used to check significant differences at $p < 0.05$.

Effect of medicinal plant extract on the prevalence of late blight disease on coated healthy tomato with plant extract

Data in Table 1 and 2 revealed that three different concentrations were made like 50, 100 and 200ppm of *Curcuma longa*, *Mentha piperita* and *Ocimum basilicum* plant extract for tomato fruit coating. Decay % of artificial inoculated and controlled tomato fruits were examined after 3 and 7 days. As the concentration of plants extract

increasing from 50 to 200ppm for fruits coating the decay % of inoculated fruits decreased continuously. Minimum decay of strawberry fruits was observed at 200ppm concentration of *Curcuma longa* (12.5%), *Mentha piperita* (8.3%) and *Ocimum basilicum* (16.66%) and after 3 days and 13%, 12% and 17.33% after 7 days respectively. Different concentrations were applied to different replications.

Table 1. Comparatively effect of different medicinal plant extract on artificial inoculated tomato fruits with *Alternaria alternata* after 3 days.

Con. of Plant extraction	Control	50ppm	100ppm	200ppm
<i>Curcuma longa</i>	57.83±1.39b	21.33±0.56d	18.33±0.78f	12.5±0.59h
<i>Mentha piperita</i>	58±1.14b	19.83±0.64d	14.66±0.56ef	8.33±0.22g
<i>Ocimum basilicum</i>	57.66±1.34b	22.5±0.67d	22±0.68e	16.66±0.34f
Average	57.83%	21.22%	18.33%	12.49%

Table 2. Comparatively effect of different medicinal plant extract on artificial inoculated tomato fruits with *Alternaria alternata* after 7 days.

Con. of Plant extraction	Control	50ppm	100ppm	200ppm
<i>Curcuma longa</i>	80.16±1.05a	26.33±0.44c	20.66±0.34e	13±0.52g
<i>Mentha piperita</i>	80.5±1.34a	22.33±0.59c	14.66±0.56de	12±0.46fg
<i>Ocimum basilicum</i>	80±1.24a	32±1.01c	25±0.56d	17.33±0.59e
Average	80.22%	26.88%	20.1%	14.33%

DISCUSSION

Alternaria alternata is widely spread and decaying pathogen of tomato fruits and also cause infection in different fruits and vegetables. Eating contaminated fruits caused serious diseases in human beings. The different plant extract was used to inhibit the growth of *Alternaria alternata* on growth media. The result indicate that plant extracts have significant potential against *Alternaria alternata*. *Curcuma longa* one of the important medicinal plant that have maximum efficacy against *Alternaria alternata*. But *Ocimum basilicum* has minimum inhibitory effect against *Alternaria alternata*. *Curcuma longa* at 200ppm inhibit 81.5% growth rate of *Alternaria alternata* but at 50ppm concentration inhibit only 31.3% after 7 days which are quite related with the recent report of Apisariyakul *et al.*, 1995, Revealed that medicinal desert plant *Curcuma longa* show fungal biomass inhibition against the filamentous fungi such as *Alternaria alternata*. *Ocimum basilicum* at 50ppm and 200ppm inhibits 32% and 70.83% growth rate respectively. Similar results were reported in recent research of Silva *et al.*, 2008, that revealed latex extracts of medicinal plant *Ocimum basilicum* have fungicidal activity (due to the presence of biologically active constituents in ethanolic extract) (Silva *et al.*, 2008). While *Ocimum basilicum* at 200ppm has minimum effect against *Alternaria alternata* and only inhibits a 70.83% growth rate. The latest report of (Fournomiti *et al.*, 2015), that showed methanolic extracts of *Ocimum basilicum* wood, bark and seeds were effective antifungal activity against *Alternaria alternata*. Both menthol and ethanol were used for making an extract of a desert plant.

CONCLUSIONS

It was observed that maximum control of *Alternaria alternata* was gained by *Curcuma longa* extract at 200ppm concentration of plant extract. So, it is suggested that *Curcuma longa* plant extract is the best treatment against *Alternaria alternata*.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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