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EFFICACY OF DESERT MEDICINAL PLANTS AGAINST POSTHARVEST LOSSES CAUSED BY BOTRYTIS CINERIA (PERS.) IN STRAWBERRY

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ABSTRACT

Botrytis cineria is a momentous pathogen of different economically important crops, causing severe diseases on more than five hundred species of plants, mainly affects fresh vegetables and fruits with economic losses of 10 to 100 billion dollars throughout the world.Grey mould, caused by *B. cineria* is considered as a major post-harvest disease of a wide range of fruits especially strawberry. Medicinal desert plants like *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera* are used as antifungal agents against post-harvest diseases of fruits caused by *B. cineria.* Different products are obtained from these desert plants and are being used for medicinal purposes and also proven good to control different diseases in fruit plants. They contain antifungal properties that cease fungal growth through different ways. These plant extracts are used because they do not contain any side effects and control the fungal growth. These medicinal plant extracts are also cheap in their cost and farmers can obtain them easily from the market and their disease controlling percentage is also the highest. *C. colocynthis* inhibits 77.9% growth rate at 200 ppm concentration. In the present research it is concentrated to control postharvest disease of strawberry fruit as during management, different plants extracts were used for fruits coating. Completely randomized design was used in lab and greenhouse experiments. For comparing the differences between the means least significant difference test was used at 5% variation. The ultimate aim of the present research is to replace synthetic fungicides by using natural products to increase shelf life of fruits, their economic value by reducing residual effects of fungicides.

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INTRODUCTION

Pakistan's climatic conditions are very good for the growth of fruits. Pakistan has inadequate facilities for processing and storage of various fruits and thereby increasing their destructibility. For the food satisfaction of rapidly increasing population a large quantity of horticultural commodities was produced in Pakistan during the last decade and its share in national Agricultural GDP is 12%. Pakistan's share in Horticultural Commodities is less than 1% in world export with a minimal worth of $0.24 billion as compared to world export that reaches up to $124 billion (FAO, 2009).

In fresh fruit, striking quantity of different antioxidants such as anthocyanin's tocopherols, phenolic compounds and carotenoids are present. Many kinds of vitamins and minerals are present in raw and fresh fruits and thus they have much importance in providing valued fitness benefits. Community organization and farming business is influenced by this to use dietary information to increase the fresh formation (Marisa, 2006). However, it is very alarming for us that due to poor postharvest practices, insufficient supervision facilities and uneven handling, a large quantity of this perishable product is being wasted. It is expected that 25-40% of the total production is lost due to poor postharvest handling which leads to bigger financial loss (GOP, 2012).

Strawberry is an important horticultural crop that is grown all around the world on more than 372000 hectares (FAO, 2019) and only in the United States, the total amount of the annually production of strawberry exceeds from US$ 2.3 billion (USDA, 2016). Strawberries are important to the human food as a source of essential micro and macronutrients. These antioxidants and vitamins promote health (Basu et al., 2014; Giampieri et al., 2015; Wang and Lin, 2000). Strawberry is a persistent herbaceous food plant with small stems and compactly spaced leaves. Strawberry helps out in the production of complex accessory and aggregate fruit comprises of a receptacle and achenes (Darrow, 1966). Achenes are minute single-seeded fruit, whereas the receptacle is known to be anatomically equivalent to tissue of floral meristem (Hollender et al., 2012).

Strawberry is infected by a number of pathogens like bacteria, fungi, nematodes and viruses. The economically most important pathogens of strawberry fruits are fungi, which can infect all parts of the plant and cause severe damage or death of the plant (Garrido et al., 2011). Among the fungal pathogens, *B. cinerea* is known as the main pathogen of postharvested strawberries in all around the world that caused the major economic losses to the industries of the strawberry. Grey mould diseaseis caused by *B. cinerea* in fruits and fruit senescing organs but also affect vegetative tissues. Under moist environment, more than 80% of strawberries and strawberry flowers can be lost if fungicides are not sprayed on plants (Ries, 1995).

Strawberry is cultivated on several areas in all around the world due to its nutritional benefits for human. It has juicy texture and strong aroma. It contains important micro and macronutrient. Strawberry production is a moneymaking business for the persons having small lands because it has pleasant and cool characteristics. Mostly it can be used in fresh form as well as different product such as jam, jellies, juices and squashes can also be made from it. World widely number of varieties are developed but in our country some of its varieties such askaroz, noor, chandler, tuftus, duglus, corona and pajaro are cultivated. A total cultivated area of strawberry in Pakistan is 78 hectares and annual production is almost 274 million tons (Mahmood et al., 2012).

In several areas of Pakistan, strawberry is cultivated but mostly it is cultivated in Charsada, Gujrat, Islamabad, Mardan, Lahore, Haripur, Karachi, Swat, and Mansehra. In Islamabad mostly Chandler varieties are grown but Pajaro, Douglus and Commander Varieties are grown only for research purpose. The optimum pH of soil required for the production of strawberry is about 4.6-6.5 (Milosevic, 1997).

In Pakistan, the production of strawberry is very low as compared to developed countries due to poor cultural practices, environmental conditions, lack of markets, poor research works etc. but one reason is also responsible for its low production and that is newly introduced crops in Pakistan. These factors are responsible for the low production of strawberry in the country as compared to other countries (Mabood., 1994).

Use of strawberry can also show a significant role in the cardiovascular disease (CVD) inhibition as strawberry contains frequent basic nutrients such as vitamins C, Potassium, fiber, several phenolic and folate (Sesso et al., 2007).

B. cineria is considered as one of most critical disease producing agent of fruits. Due to this pathogen,

economic losses throughout the world reach up to $10 billion each year (Weiberg et al., 2013). *B. cineria* is scientifically and economically important and grouped as most widespread plant pathogen. These pathogens are assimilated and colonized through insemination and adhesion on the surface of plants whereas characteristics like sensing and perceiving of host surface along with hydrophobicity and sugar sources assume crucial (Doehlemann et al., 2006).

Fungicides are commonly used to control postharvest diseases caused by pathogens but these chemical agents have adverse effects on human health and all other living organisms. Moreover, during storage due to short time interval among analysis and utilization of horticultural products, the use of these fungicides becomes 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).

To introduce new crops and protecting their storage products during postharvest, control strategies are important. With using of small amount of conservative fungicide or even not using synthetic fungicide at all, has been observed due to the significant reduction in pesticide efficiency along with amazing decrease of marketable fungicide as well as latest concern about the environment and human effects of used synthetic fungicides due to the recent European laws (Kim et al., 2003). The fruit losses which occur during harvesting and storage can be decreased by minimizing mechanical injury of fruits during harvesting, their proper storage under modified safe treatment of fruits which are done before and after harvesting and under safe atmosphere (Janisiewicz, 1988; Janisiewicz et al., 1994).

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 reserve food and to control pathogens (Perez-Pacheco et al., 2004). Plant extracts from different plants have been examined to check their activities in order to control fungal pathogens (Daferera et al., 2003), and to remove harmful oxidizing agents (Skerget et al., 2004).

Variety of antifungal agents are produced within the plant body which protect them against pathogenic microorganisms and make them resistant against a number of fungal pathogens (Mysore and Ryu, 2004). Plant extracts have been used to control post-harvest pathogen as natural sources (Cárdenas-Ortega et al., 2005). Plant extract consists of mixture of complex organic compounds such as monoterpenes, sesquiterpines and phenylpropanoids to protect them against attack of pathogens (Fujita and Kubo, 2004). Due to complication in chemical characteristics of plant extracts, resistance against fungal attacks is higher because both active and inactive molecules contribute in antifungal activity (Guerreiro *et al*., 2015). Assimilation of these plant extracts into edible coating is more adequate measure to control fungal problems by decreasing the dispersion action and by increasing the concentration of effective particles on fruit surface (Aloui et al., 2014).

It is necessary to develop new antifungal product in order to reduce economic losses and to produce healthier food (Burt and Reinders, 2003). Moreover, the major reason of disease dispersion in agriculture is resistivity of pathogens against fungicides. To control plant pathogens, the need is to introduce new alternative agents. Organic compounds alkaloids, phenols and terpenes produced by the plants emerge as plant protector against pathogens attack (Benner, 1993). Normally, natural plant extracts have ability to control fungal pathogens and also are considered as safer for plants and environment. From the last few years' interest in the advancement of non-toxic antifungal agents like plant extracts are used to control plant pathogens have been developed. Scientific investigation of plants which are medicinally valuable and have potential against microbial diseases is important (Kordali et al., 2003).

This study was focused to compare the efficacy of distinct desert plants like *Citrullus colocynthis, Capparis decidua* and *C. procera* growing in the Cholistan desert of Pakistan and examined the effect of the main fungal pathogen *B. cineria* on some economically important fruits especially strawberry.

MATERIALS AND METHODS

Collection of infected fruit samples

The infected samples of strawberry fruits were collected from different fruit shops, markets located in Faisalabad in the polythene zipper bags and proper labeling with permanent marker were taken to laboratory of

department of plant pathology, Faculty of Agriculture, University of Agriculture Faisalabad. The infected samples of infected fruits were collected and signs and symptoms of *B. cineria* were observed.

Isolation of fungus

The fungus was isolated and observed from different infected fruit samples by following the method. About 2-3 cm rotted portion of infected samples were separated and cut into small pieces. Then surface sterilized with 70% of ethyl alcohol afterward treated sample rinsed two times with water and then the samples were dried with sterilized filter paper. Small pieces of diseased parts were transferred on to the surface of PDA plates. For removing and inserting diseased parts of infected samples needle, scalpels, and scissors were sterilized by dipping in the methylated spirit blazing many time. The inoculated petri plates were sealed with paraffin and incubated at 28 ℃ for 36 hours. Within 24-36 hours, fungal colonies appeared on PDA media.

Identification and purification of isolated fungi

The fungus was detected and characterized. Pure culture of *B. cineria* was prepared for further usage. Purification of fungus was done in laminar air flow chamber. In this way pure culture was obtained.

Pathogenicity Test

In vitro pathogenicity test of isolated fungi was carried out. In laboratory, pure culture of *B. cineria* was produced on PDA. Healthy fruits were surface sterilized with 70% ethanol to make it free from fruits surface contamination. Then rinsed thoroughly with double distilled water and filter paper used to minimize the excessive wetness. After that fruits were inoculated with the help of inoculated needle and were packed in polythene zipper bags and placed in the incubator at 27±2 ºC. After 2 days, symptoms were observed on the fruits surface. The untreated fruits were considered as control.

Collection and Preparation of plant extracts

The desert plants (*Calotrpis procera, Citrullus colocynthis* and *Capparis decidua*) were collected from the Cholistan desert and brought to the laboratory of Department of Plant Pathology for further usage. For the process of preparation of extract, plant parts cut into small pieces separately, and ground in the blender (food processor). Different plant parts were used to make extracts like leaves, roots and stems etc. First of all, prepared plants extract were passed through uncontaminated muslin cloth and then passed through filter paper. Different concentrations of plants extracts were used against *B. cineria.*

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 sterile distilled water. Properly cleaned plant materials were dried in shade. Air dried plant materials cuttings were powdered using powdering mill to 50 mesh sizes. Seeds were separated by depulping 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 (300 g each) were extracted with methanol in a soxhlet extractor for 8 to 10 hours. The process was run until the decolourisation of the solvent, after which the extract was filtered with Whatman filter paper (No.1) and the filtrates were concentrated using a rotary evaporator. The extracts were then evaporated to dryness over 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 kept in labeled sterile specimen bottles.

***In vitro* evaluation of different desert medicinal plant extracts against *Botrytis cineria* by using poisoned food technique**

Efficacy of plant extracts against *B. cineria* was tested by using poisoned medium technique using PDA media. Different concentrations of plant extracts were mixed in sterile molten PDA media. For inoculation plug of pure mycelium was taken from 4 days old culture and placed in the center of Petri plates in inverted form. After inoculation, Petri plates were incubated at 27+2 ºC. According to Cakir et al. (2005) mycelium growth inhibition of treated samples against control was calculated

$$\text{Inhibition } \% = \frac{C - T}{C} \times 100$$

Where,

C = Growth of mycelia in controlled plate

T = Growth of mycelia in treated plates

Management through plant extract

Efficacy of desert plant extracts through coating on artificially inoculated fruits

To inoculate strawberry fruits, 10 days old culture of *B. cineria* was taken for conidial suspension preparation. Concentration of conidia was adjusted by using heamocytometer. Before inoculation, fruit surface was

sterilized with the help of 95% ethyl alcohol. Fruits were inoculated by dropping conidial suspension on fruit surface. After inoculation, fruits were covered with the help of plastic bag for a day. After that, infected strawberry was removed and placed in tray at same condition. Solutions were prepared by mixing plant extract into distilled water. Treated and controlled fruits were dipped into solution for few seconds and again placed into plastic bags at room temperature.

Statistical analysis

In vitro grey mould disease management through number of plants extracts was carried out in laboratory condition under complete randomized design. Data from all the experiments were analyzed by using analysis of variance and means of were compared by least significance difference (LSD) test (0.05).

RESULTS AND DISCUSSION

Effect of medicinal desert plant extracts on the radial growth of *Botrytis cineria*

Statistical data have been shown in Figure 1. Three different concentrations were made like 50, 100 and 200 ppm of *Capparis decidua, Calotropis procera and C. colocynthis* plant extracts*.* Fungal growth was inhibited continuously with the increasing concentration of medicinal desert plant extract from 50 ppm to 200 ppm. Maximum inhibition of *B. cinerea* was observed at 200 ppm concentration of *C. decidua* (46.33%), *C. procera* (66.83%) and *C. colocynthis* (66.33%) after 3 days and 54.03%, 69.33% and 77.33% after 7 days respectively. Different concentrations were applied on different replications*.*

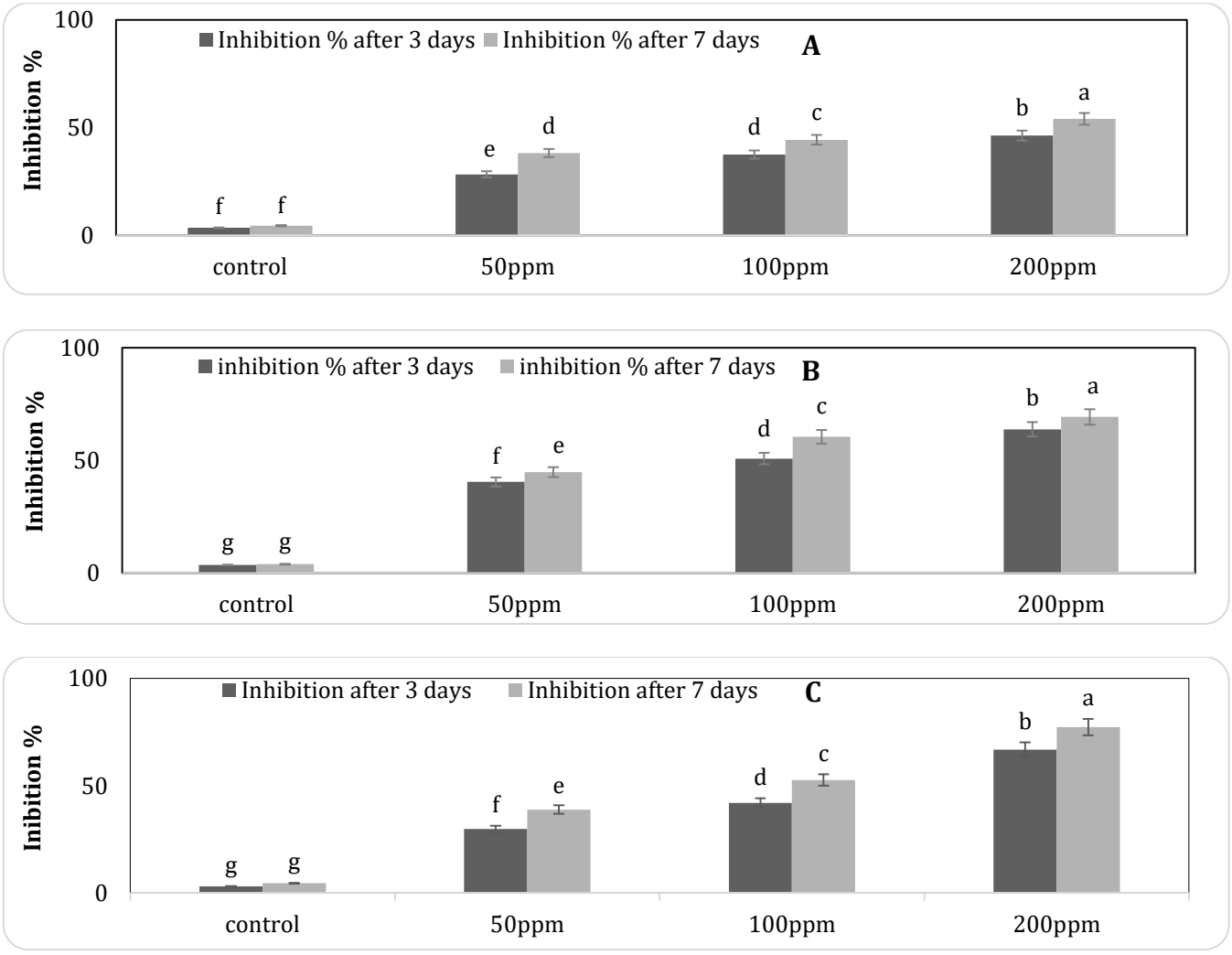

Figure 1: Effect of medicinal desert plant extracts of *C. decidua* (A), *C. procera* (B) and *C. colocynthis* (C) on the radial growth of *Botrytis cineria*.

These graph average values are the means of three replications. LSD test was used to check significant differences at p<0.05.

Effect of medicinal desert plant extracts on the incidence of grey mould disease on coated strawberry fruits

Data in Tables 1 and 2 revealed that three different concentrations were made like 50, 100 and 200 ppm of *Capparis decidua, Calotropis procera and C. colocynthis* plant extract for fruit (strawberry) coating. Decay percentage of artificial inoculated and controlled strawberry fruits were examined after 3 and 7 days. As the concentration of plants extracts increased from 50 to 200 ppm for fruits coating, the decay percentage of inoculated fruits decreased continuously. Minimum decay of strawberry fruits was observed at 200 ppm concentration of *C. colocynthis* (5%), *C. procera* (9.5%) and *C. decidua* (26.33%) and after 3 days 9.66%, 10.33% and 32.0% after 7 days, respectively. Different concentrations were applied on different replications.

Table 1: Comparative effect of different medicinal plant extracts on artificial inoculated strawberry fruits with *Botrytis cineria* after 3 days.

Con. of Plant extraction	Control	50 ppm	100 ppm	200 ppm
C. colocynthis	45.5±1.39b	18.66±0.56d	12.33±0.78f	5±0.59h
Calotropis procera	47.43±1.14b	21.26±0.64d	17.33±0.56ef	9.5±0.22g
Capparis decidua	49.16±1.34b	37±0.67d	30.66±0.68e	26.33±0.34f
Average	47.36	25.64	20.10	14.27

Table 2: Comparative effect of different medicinal plant extracts on artificial inoculated strawberry fruits with *Botrytis cineria* after 7 days.

Con. of Plant extraction	Control	50ppm	100ppm	200ppm
C. colocynthis	76.43±1.05a	20.5±0.44c	15.33±0.34e	9.66±0.52g
Calotropisprocera	74.65±1.34a	21±0.59c	16.36±0.56de	10.33±0.46fg
Capparis decidua	75.34±1.24a	43.33±1.01c	38.33±0.56d	32±0.59e
Average	75.47	28.27	22.92	17.33

Botrytis cineria is widely spread and decaying pathogen of different fruits such as strawberry, apple and grapes and also cause infection in different vegetables. Eating contaminated fruits caused serious diseases in human beings. Different plant extracts were used to inhibit the growth of *B. cineria* on growth media. The result indicated that plant extracts have significant potential against *B. cineria*. *Citrullus colocynthis*is is one of the important medicinal desert plants that had maximum efficacy against *B. cineria.* However, *Capparis decidua* had the minimum inhibitory affect against *B. cineria*. *C. colosynthis* at 200 ppm inhibited 77.33% growth rate of *Botrytis cineria* after 7 days, which are quite related, with the recent report of Al-Dhabi et al. (2015) revealed that medicinal desert plant *C. colocynthis* showed fungal biomass inhibition against the filamentous fungi such as *B. cinerea. Calotropis procera* at 200 ppm inhibited 69.33% growth rate. Similar results were reported in recent research of Saratha and Subramanian (2010) who revealed latex extracts of medicinal plant *Calotropis procera* had fungicidal activity (due to the presence of biologically active constituents in ethanolic extract of calotropis latex). On the other hand, *Capparis decidua* at 200 ppm had minimum effect against *Botrytis cineria* and only inhibited 54.03% growth rate. Latest reports showed that methanolic extracts of *C. decidua* wood, bark and seeds were effective against *Botrytis cineria* (Abdalrahman et al., 2016; Tlili et al., 2011; Tripathi et al., 2015).

CONCLUSION

It was observed, that the maximum control of *Botrytis cineria* was gained by *Citrullus colocynthis* extract at 200 ppm concentration. Therefore, it is suggested that *C. colocynthis* plant extract is the best treatment against *B. cineria.*

AUTHORS' CONTRIBUTION

MTM, WA, MAZ and MSUR designed the study, MTM, FR and SM conducted experiments and recorded data, ARK analyzed the data, MTM and ARK wrote the manuscript and all the authors read and apporved it.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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