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SUSTAINABLE CONTROL OF TOMATO LEAF BLIGHT DISEASE CAUSED BY ALTERNARIA ALTERNATA AND CURVULARIA LUNATA IN DISTRICT KAMBER SHAHDADKOT, SINDH, PAKISTAN

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Abstract

Family Solanaceae has achieved illustrious value in the world because of its supreme plant members. Tomato (Solanum lycopersicum L.) has the credited with second pillar of the family. It is cultivated throughout the world for fresh fruits and different processed products. Fungi are most common pathogens on tomato plants. They cause number of diseases like early and late blights, leaf molds, and septoria leaf spot. To control these fungal diseases fungicides are used by people for a long time. From the one side these fungicides control or reduce the fungal infections but from another side they create health and many environmental problems. Present attention has been targeted on the use of natural products of plants origin as proxy to laboratory prepared fungicides. The natural plant extracts are not only effective but also safe to human health and are biodegradable.

To avoid the problems caused by fungicides natural control of fungal diseases through various botanical extracts has been applied. In vivo use of plant leaves extracts shown significant results against growth and development of fungal mycelia on tomato plants. The research was presided over to study the antifungal activity of various plant leaves extracts i. e. neem, basil, eucalyptus and garlic beside fungicides. Total five aqueous solutions of plant extracts were prepared and tagged with ABC letters. The tomato plants were cultivated in six field plots of 4 by 4 meters. The selected fungi members i.e. Alternaria alternata and Curvularia lunata were inoculated on one month old fresh leaves of tomato plants of respective plots. Having infections started the treatments of various extracts were sprayed time to time up-to fruits ripening while the plants of control plot were left free. At the over the course of experiment, aqueous extract of neem tree leaves expressed highest control 53% followed by the basil 47%, garlic 43% eucalyptus 38%, mixed 49% comparatively against controlled plants. Apparently the fungi shows difference in its response to the different concentration of extracts but subsequently growth inhibits with the increase of concentration.

This study aim to examine the antifungal properties of different plant leaves extracts, to make the tomato fruits safe and protect the land from effects of various fungicides.

Keywords: *Fungicides, Azadirachta indica, Osimum basilicum, Meliaceae, tomato, basil, and eucalyptus.*

Introduction

A plant that is grown all over the world and is highly valued for the extraordinary flavor, colour, taste, and nutritional content of its fruit is the tomato plant (*Solanum lycopersicum* L.) the member of the Solanaceae family, further more referred to as the family of Nightshades (Ofori *et al*., 2022). Tomatoes are recognized as the second most consumed vegetable crop globally, trailing only behind potatoes (Iquebal *et al*., 2013). The versatility of tomatoes is evident in their various culinary applications, as they are utilized in the preparation of dishes such as sauces, pastes, ketchup, salads, and beverages (Nugroho *et al*., 2019; Poussio *et al*., 2022). Beyond their culinary uses, tomatoes are nutritionally rich, providing essential antioxidants, vitamins A, C, E), (Kumar *et al*., 2017), and minerals such as calcium, iron, and phosphorus (Meena and Bahadur, 2014). Additionally, tomatoes are the robust spring of carotenoids and lycopene, a renowned source for its potential role in preventing cancer, reducing cardiovascular risks, and slowing down cellular aging (Amri, 2013).

In Pakistan, familiar tomato cultivars, including Riogrande, Roma, and Money Maker were imported from America and Europe (Khokhar, 2013). These varieties are cultivated across various provinces in Pakistan (Memon, 2013). Globally, the total production of tomatoes amounts to 161.7 million metric tons, with an estimated value of $59 billion. The widespread cultivation and nutritional richness of tomatoes underscore their importance in both culinary and health contexts (Adhikari *et al*., 2017). Globally production of tomatoes 42. 2 Million Metric tons, 2023 by Allisa Dillon. About 63000 hectares of land was used for tomato cultivation in Pakistan, and yield about 600,000 tonnes of tomatoes all inclusive but despite that, the Sindh province used 14,000 hectares and produced virtually 163,000 tonnes of tomatoes at same time (Wahid *et al*., 2017). The proliferation of food industries within a country is anticipated to amplify the demand for tomatoes, particularly in the preparation of tomato made foods (Tahir *et al*., 2012).

However, in Pakistan, in comparison to other developed countries, the yield of tomato crops is hindered by various perilous diseases caused by nematodes, fungi, bacteria, and viruses (Raza *et al*., 2016). The postharvest time is particularly susceptible to fungal diseases, contributing to a potential. The whole tomato yield is lost by 50% (Ippolito *et al*., 2005). Compounding these challenges are factors such as the scarcity of quality seeds and exposure to abiotic stresses, namely salinity and drought, which significantly contribute to the reduction in tomato crop production. Several fungal pathogens have been reported to not only devastate but also diminish the overall production of tomato crops.

Leaf blight, a prevalent affliction among tomato plants, is predominantly caused by the fungi *Curvularia lunata* and *Alternaria alternata*. This fungal disease poses a significant threat to the vitality of both tomato and potato crops. Initially manifesting on the lower leaves as the plants mature, early signs of leaf blight emerge in the form of small, dark lesions characterized by concentric circles, often encircled by a yellowing zone (Kokaeva *et al*., 2018). Given conducive environmental conditions, the disease proliferates throughout the plant, affecting leaves, stems, branches, and fruits, ultimately resulting in leaf detachment, branch desiccation, and premature fruit loss. Yield losses due to leaf blight can be substantial, ranging from 50 to 86% (Abdelfatah *et al*., 2021; Akhtar *et al*., 2004). According to earlier reports, *Alternaria alternata* frequently causes post-harvest losses in tomatoes and manifests the signs of leaf blight. The late blight symptom of nuts, which affects fruit and foliages and is typified by the formation of huge necrotic spots or lesions that finally coalesce and damage the entire leaf, defoliates the tree, is another symptom of *Alternaria's* attack on vegetables (Sobhi *et al*., 2023).

To address the impact of different plant extracts on fungal species, four specific plants have been selected for study. In present-day tomato plant cultivation, a significant challenge arises from the limited selection of effective agents to combat phytopathogenic fungi, coupled with the emergence of fungal resistance to existing treatments to tackle this issue, ongoing research is fervently exploring environmentally sustainable chemicals that not only preserve our ecosystems but also exhibit potent antibiotic properties to ensure robust crop protection (Martinko *et al*., 2022). This research aims to explore potential solutions to reduce the destructive effects of fungal pathogens on tomato crop, considering the worth of tomatoes in the industries of food and the agricultural challenges faced in Pakistan (Zhu *et al*., 2014).

The Neem Tree (*Azadirachta indica* L.), Being part of the family Meliaceae, is abundantly distributed in tropical and semitropical regions such as Pakistan, India, Bangladesh, and Nepal. This fast-growing woody tree can reach heights of 20 to 30 meters, characterized by compound leaves with approximately 15 leaflets. Its green drupe fruits undergo a transformation to golden yellow upon ripening, typically occurring between June and August (Alzohairy, 2016). Recognized for its medicinal properties, the Neem plant has garnered attention in the scientific community for its efficacy against fungal growth (Ezeonu *et al*., 2018).

Extensive research has revealed that Neem tree extracts possess potent antibiotic properties, showcasing notable antiviral, antibacterial, and antifungal characteristics. The fungicidal attributes of Neem extracts have demonstrated significant promise, leading to a substantial reduction in the conidial germination of various fungi. The multifaceted therapeutic potential of the Neem tree, validated by scientific studies, underscores its importance in traditional medicine and highlights its promising role in invading fungal contaminations (Quraishi *et al*., 2018; Bhowmik *et al*., 2010).

Basil (*Osimum basilicum* L.) is ever shine herb plant, bonding to lamiaceae family. It is growing in numerous countries of the world (Moghaddam *et al*., 2011). In the field of medical and agriculture, basil extracts has been described to hold back the development and growth of fungal pathogens (Bansod and Rai, 2008). More ever previously reported that aqueous extract of *Osimum basilicum* significantly reduced the early blight incidence on tomatoes originated by *Alternaria solani* under field and green house conditions (Nashwa *et al*., 2012). The ingredients of *Osimum basilicum* completely inhibit the growth of *Rhizopus stolonifer*, a postharvest fungal pathogen in vapor phase method (Abdollahi *et al*., 2011). Considered as a medicinal plant has been already tested successfully against soil borne and seed borne fungi (Diakalia *et al*., 2018).

Eucalyptus (*Eucalyptus globulus* L.) holds a prominent position within the Myrtaceae botanical family and is widely distributed globally (Ghareeb *et al*., 2018). Extensive pharmacological and phytochemical investigations have highlighted the antifungal capability of various extracts derived from Eucalyptus camaldulensis. Methanolic extracts of *E. camaldulensis* have demonstrated activity against *Alternaria alternata*, a plants pathogenic fungus known for creating leaf spot and various diseases across more than 381 host plants (Singh *et al*., 2014). Notably, essential oils from *E. camaldulensis* further more the organic aqueous extracts, have been subject to research, revealing significant antifungal job against a broad spectrum of phytopathogenic fungi with economic importance.

The utilization of eucalyptus leaf extracts as a medicinal remedy for fungal infections traces back to the indigenous practices of the Australian people. This historical use underscores the long-standing recognition of the antifungal properties inherent in Eucalyptus, further supported by contemporary scientific investigations. The multifaceted contribution of Eucalyptus in combating phytopathogenic fungi not only validates its traditional medicinal applications but also highlight its potential as a value resource in modern pharmacology and agriculture (Gilles *et al*., 2010).

Garlic (*Allium sativum* L.) is the biennial herb plant belonging to the Alliaceae family, which comprises 30 genera and around 600 species (Agil and Azike1, 2019). This versatile vegetable crop holds significant agricultural importance and is cultivated in various regions, including Argentina, Nigeria, India, Korea, Thailand, Italy, Mexico, USA, and Spain, (Medina and Garcia, 2007). Typically, a garlic plant can reach heights of 30-90 cm, with its below-ground bulb being the primary component. The bulb is composed of segments known as cloves, with each bulb containing 6-12 cloves. The distinctive aroma of garlic is attributed to its sulfur-containing constituents, which are also recognized for their medicinal properties (Davies, 2012).

Garlic has a rich history of use in both culinary and medicinal applications. The antifungal properties of garlic are primarily attributed to allicin and allicin-derived compounds. A study demonstrated that pure allicin exhibited antifungal properties, and the removal of allicin led to a decrease in antifungal activity (Harris *et al*., 2001). Numerous studies have provided evidence supporting the antimicrobial effects of garlic (Martin *et al*., 2003). Furthermore, garlic extract has been found to mitigate early blight disease in tomatoes and completely inhibit the mycelial growth of A. flavus, subsequently suppressing aflatoxin secretion (Ikon *et al*., 2017). The multifaceted properties of garlic make it a valuable asset not only in the culinary world but also in agriculture and medicine.

This study have aim to determine the antifungal efficacy of neem, basil, eucalyptus and garlic aqueous extract to control and reduce the fungal growth as well as spore germination on tomato plant body.

Fungicides

Fungicides encompass chemical compounds or biological agents designed to eradicate parasitic fungi or their spores. The detrimental impact of fungi on agriculture can lead to significant losses in yield, quality, and overall profitability (Rouabhi, 2010). Several fungicides have been reported effective against various fungi (Keyser *et al*., 2017) but along with this, fungicides are causing number of effects in environment and on health of man. However human health environmental and food safety issues must be considered to choose a method for controlling the diseases. So for that environmental-friendly efforts should be taken to control diseases by using botanical pesticides (Nugroho *et al*., 2019). Recent endeavors have thus concentrated on developing environmentally friendly, durable, and efficient bio-control techniques and treatments for managing plant diseases (Joseph *et al*., 2017). The utilization of plant extracts has proven to be both eco-friendly and effective against various plant pathogens (Chohan *et al*., 2015). Ongoing research involves the evaluation of extracts from different plants to identify safe alternative control methods that are harmless to men and the environment (Emeasor *et al*., 2002). Natural products, known for their non-toxic nature, easy biodegradability, and safety towards non desirable organisms and natural enemies, do not leave residues in food products (Kimani, 2014). A multitude of plant species has been documented to hold natural chemicals that exhibit toxicity towards numerous fungal pathogens (Goussous *et al*., 2010).

Hence, this current investigation aims to assess the extent of blight severity on tomatoes and to assess the effectiveness of leaf extracts derived from Neem, eucalyptus, basil, and garlic in mitigating fungal diseases affecting tomato plants.

Materials and methods

This study was done in Botany lab, Centre for Biodiversity and Conservation Shah Abdul Latif University Khairpur and field areas of tomato crops of District Kamber Shahdadkot Sindh Pakistan.

Isolation.

Tomato plant leaves infected by *Curvularia lunata* and *Alternaria alternata* were observed and collected in polythene bags separately and brought to Botany lab at Shah Abdul Latif University Khairpur for upcoming steps of isolation and identification. All the samples were washed with tape water and dried in natural environment. Later the samples were cut into small pieces of 2 cm and soaked into the 1% sodium hypochlorite for one minute to be surface sterilized (Nurhaida *et al*., 2019). All the samples were cultured separately into the petri plates having Potato Dextrose Agar (PDA) that is most common and best media for growth and development of fungal mycelia (Aujla and Paulitz, 2017). The plates were set in incubator having room temperature 25 ^{0}C to 35 ^{0}C for growth and development of fungal colonies. After three days fungi were appeared at the point of inoculation and spores were formed within five to seven days.

Identification.

Observed and noted the key morphological characteristics of the *Curvularia lunata* and *Alternaria alternata*, such as the arrangement, form and colour of the spores, type of mycelium, the growth process, and the morphology of the cultures on PDA media.

Morphological characterstics of cultures and nature of growth.

At the first instance the population of *Curvularia lunata* were appeared with white mycelial colour to dark brown onto the potato Dextrose Agar medium's surface, but when they reached at desired growth stage, they turned grayish black. The colonies of *Alternaria alternata* cultures in petri plates were appeared dark olivaceous brown to dark black brown on the PDA media's surface (Alex *et al*., 2013).

Microscopy of mycelia and spores/ conidia

Based on type and nature, the mycelia of *Curvularia lunata* exhibited branching, hyaline characteristics, and septate structures. Conidia appeared clavate or widely ellipsoidal, presenting a brownish hue with smooth walls, septation, typically comprising four cells, slightly curved, and arranged in sympodial orders. The central cells of conidia appeared broader, thicker, and darker compared to the sub-terminal cells, delineating distinct morphological features of fungal development (Kibemo, 2017; Zheng *et al*., 2014). Microscopic examination of *Alternaria alternata* exhibited septate hyphae and primary conidiophores that were either straight or bent and produced long chains of conidia. The light brown conidia had tapered apices and were typically formed in chains, but sometimes they formed separately in a muriform style. Obclavate, long, ellipsoid, small to reasonably sized, septate, straight, pale to dark brown to olivaceous green, and velvety were the conidia's features. With one or a few conidiogenous loci apical or laterally, secondary conidiophores can occasionally arise (Woudenberg *et al*., 2013; Lawrence *et al*., 2013).

Following careful examination, the species have been positively identified as *Alternaria alternata* and *Curvularia lunata*, each of which has unique traits. This conclusion was reached after a thorough investigation that guaranteed the findings' originality and legitimacy.

Collection of botanicals

Leaves of Neem tree (*Azadirachta indica* L.), Basil (*Osimum basilicum* L.) and Eucalyptus (*Eucalyptus globulus* L.) were collected from Shah Abdul Latif University Khairpur while Garlic (*Allium sativum* L.) was purchased from market of Kamber city. The gathered leaves underwent a through cleansing process using distilled water, followed by sun-drying and subsequent individual pulverization using an electric grinder machine. The garlic cloves extracts were obtained by crushing process. The powdered materials of all three plant leaves and garlic cloves extraction were stored separately in glass jars i.e. jar A, B, C, D and E. Jar A had Neem leaves powder, Jar B Basil, jar C, Eucalyptus, jar D Garlic cloves extraction and jar E contain mixture of all botanicals.

Extracts preparation

The aqueous leaf extracts of the samples were meticulously prepared by immersing the respective powders in distilled water at a ratio of 10 g powder to 100 ml of distilled water, allowing for a 24-hour soaking period. Following this duration, the suspensions underwent filtration using Whatman's filter paper. Subsequently, 30% concentrations of each extract were meticulously formulated using distilled sterilized water. This method ensured the extraction of the desired components while maintaining the integrity of the extracts (Joseph *et al*., 2017).

Experimental design and treatment applications

For the experimental work Roma tomato variety was cultivated in the field. To conduct this experimental work, field plots (4 by 4 m) based on three rows were prepared with 0.5 meter distance. Twelve tomato plants were cultivated in each plot having four plants in each row. A total of twelve plots were employed in three groups i. e. Group A and group B as replications for each treatment. The young leaves of group A plants were inoculated with 5mm disc of pure culture of *Curvularia lunata* and the plants of group B with *Alternaria alternata*. The tomato plants of group C as controlled plants were inoculated with both fungal species separately and were left free from any botanical extract treatment. Within few days both fungal species started infection on all inoculated plant leaves but onset of symptoms appearance the treatment was started with respective plants extracts on the infected parts of plants except the plants of group C.

Treatments

Total ten treatments were applied from the age of one month up to the fruits ripening. Plants of all plots were treated with their particular plant extracts while the plants of control were remained free of any treatment.

Results

The effects of different plant leaves extracts like neem, basil, eucalyptus and garlic shown significant results against growth and development of fungal mycelia on tomato plant leaves. The percentage of inhibition of expansion of fungal mycelia radially on tomato plant leaves medicated by all leaf extracts is given in Table 01. The leave extracts of all given plants remarkably inhibited the development of fungal mycelia. The percentage of inhibition by leaf extracts is varied by different leaf extracts. The inhibition property of neem leaves extract is higher that is 53% while the inhibition property of eucalyptus leaves extracts has been observed lower which is 38%. The control could not express the suppression of the emergence of fungi and development on tomato plants.

Table 1The percentage of growth inhibition of fungal species by leaf extracts of neem, basil, eucalyptus and garlic on tomato plants

S. NO.	NAME OF PLANT	EXTRACT	INHIBITION PERCENTAGE
01	Neem (*Azadirachta indica* L.)	Aqueous	53%
02	Basil (*Osimum basilicum* L.)	Aqueous	47%
03	Eucalyptus (*Eucalyptus globulus* L.)	Aqueous	38%
04	Garlic (*Allium sativum* L.)	Aqueous	43%
05	Mixed all plant's leaves extracts	Aqueous	49%
06	Control	No treament	00%

Discussion

The efficacy of neem, basil, eucalyptus and garlic extracts against fungal species *Alternaria alternata* as well as *Curvularia lunata* which induce leaf blight were used in vivo in the field areas of tomato crops. The results were observed with significant inhibition of radial growth of the fungal species with different percentage from

one to another. The neem tree leaf extraction was dominant among all others while eucalyptus observed lowest percentage of inhibition. All the leaf extracts were effective against fungal species on tomato plants. Having all these positive results it was confirmed that the leaf extracts of neem, basil, eucalyptus, and garlic extracts have demonstrated remarkable efficacy against leaf blight disease. The antifungal properties of neem (*Azadirachta indica* L.) are well-established, offering a natural defense against fungal infections. Basil extracts, known for their potent antimicrobial characteristics, exhibit effectiveness in combating diverse fungal strains. Eucalyptus extracts demonstrate antifungal potential, particularly against economically significant phytopathogenic fungi. Garlic extracts, containing allicin and other sulfur compounds, contribute to strong antifungal activity. The collective impact of these plant extracts highlights their utility as natural agents in the fight against fungal pathogens, illustrating their potential in agriculture and medicine.
Another activity noticed was that the leaf extracts of the selected plants were not toxic to tomato plants and did not subscribe to soil pollution.

Conclusion

Neem, basil, eucalyptus and garlic are rich source of ingredients possessing therapeutic implications used traditionally worldwide but specially in Asian countries as they are easily available all the time. These valuable plants emerge as potent allies in combating fungal pathogens on tomato crops. Neem, with its antifungal properties, provides a natural defense, while basil's strong antimicrobial characteristics contribute to fungal resistance. Eucalyptus, known for its efficacy against phytopathogenic fungi, proves valuable in protecting tomatoes. Garlic, containing allicin and sulfur compounds, showcases robust antifungal activity. Together, these natural extracts offer a multifaceted approach, demonstrating their utility in safeguarding tomato plants against fungal threats. Their collective effectiveness signifies their potential role in sustainable and eco-friendly strategies for tomato cultivation but majority of the agriculturist are innocent from the beneficial effects of these plants against fungal infections. Through the study and survey available in favor of neem, basil, eucalyptus and garlic plants that they have significant effects against fungal diseases. Various studies like clinical and field based have confirmed the significant results of the mentioned plant extracts against number of human and plant fungal diseases. Regarding this further extensive research must be done on these plants extracts for betterment of human health and safe environment.

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