



Research Article

In Vitro Efficacy of Some Medicinal Plant-Extracts Against *Curvularia lunata* Causing Blight Disease of *Solanum nigrum* L.

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Received: June 29, 2024

Accepted: August 21, 2024

Online Published: August 21, 2024



Abstract: Fungi are both harmful and beneficial to plants they are the causative agents of many plant diseases through the use of diverse strategies. Sometimes they kill their host plants thus there is a need to control the fungi for proper growth and development of plants. *Curvularia lunata* (Wakker) Boedijn is a causative pathogen of many medicinal plant diseases, especially leaf spot and blight disease of *Solanum nigrum* L. The present study was conducted to determine the inhibitory effects of six selected medicinal plant extracts viz. *Ocimum sanctum* L., *Solanum melongena* L., *Bergera koenigii* L., *Calotropis gigantea* (L.) W.T.Aiton, *Justicia adhatoda* L., *Andrographis paniculata* (Burm.f.) Wall. ex Nees against mycelial growth of *Curvularia lunata*. Both ethanolic extract and aqueous extract show significant inhibition of tested pathogenic fungi as compared to control.

Citation:

Khatun, S. (2024). In vitro efficacy of some medicinal plant-extracts against *Curvularia lunata* causing blight disease of *Solanum nigrum* L. International Journal of Nature and Life Sciences, 8 (2), 125-131.

Keywords: Antifungal activity; *Curvularia lunata*; Medicinal plants; Phyto-extract.

1. Introduction

Plants harbour different habitats for various microorganisms that are present below or above the ground parts of the plants. These plant-associated microorganisms play an important role in regulating many biological processes that are related to plant health and growth. One or more parts of the plants that can be used for therapeutic purposes are known as medicinal plants. It has been used as traditional medicine for centuries in different countries as it becomes exhibit antifungal and antibacterial activities (Shekhawat and Vijayvergia, 2010). *Solanum nigrum* L. is an important medicinal plant belonging to the family Solanaceae. It is commonly called blackberry nightshade (Grubb and Raser-Rowland, 2012). *S.nigrum* is used in traditional medicine in India. Infusions are used in dysentery, stomach complaints and fever (Jain, 1968). This medicinal herb has a variety of medicinal activities like anti-oxidative, anti-cancerous detoxifying, neurological and anti-ulcerogenic activity (Ali et al., 2023).

Fungi are natural decomposers of the ecosystem. They prefer low pH or slightly acidic soil where the soil is undisturbed. Soil fungi constitute 10 to 20% of soil rhizosphere. Generally, more bacteria are present in the soil than fungi, but due to their larger size, fungi dominate the total biomass in healthy soil (Hoorman, 2011). They are beneficial for plants but some fungi are harmful as they cause many plant diseases. They reduce the production and quality of plant products (Haider et al., 2020). The genus of *Curvularia lunata* (Wakker) Boedijn belongs to the family Dothideomycetes is filamentous, dematiaceous, pale black or brown pathogenic fungi with



cylindrical and slightly curved conidia which are responsible for several diseases of plants, animals and humans (Odyuo et al., 2018). These microorganisms colonize in the soil and vegetation and spread through airborne spores. They are both phytopathogenic and zoopathogenic (Wilhelmus and Jones, 2001). Mainly in tropical and subtropical areas most species of *Curvularia* are facultative pathogens of plants and cereals. Some common plant occurring diseases are wound infection; skin ulceration, onchomycosis, subcutaneous mycetoma, leaf spots, blights, grain deformation, grain discoloration, and even root rot (Mishra et al., 2009). Other infections include dialysis-related peritonitis, postsurgical endocarditis, invasive and allergic sinusitis and bronchopulmonary disease. Wilhelmus and Jones reported the first human disease caused by *Curvularia* was an infection of the Cornea. Conjunctivitis, dacryocystitis, sino-orbital cellulitis and endophthalmitis are also responsible for some species of *Curvularia* (Wilhelmus and Jones, 2001). *Curvularia* leaf spot disease of maize in China caused by *C. lunata* (Wang et al., 2022). It causes economic losses in China. *C. lunata* causing leaf spot on *Oryza sativa* in Sabah, Malaysian Borneo is first reported by Ying et al. (2023). *C. lunata* causing leaf spot disease of banana from Raiganj, West Bengal, India is first reported by Chowhan and Chakraborty (2023).

Many synthetic fungicides are widely used to control diseases but have adverse effects on plant and human health. These fungicides cause hazardous effects on aquatic and terrestrial ecosystems. The application of these synthetic chemicals may be responsible for the loss of microbes present in soil and the loss of biodiversity. So biofungicides that become eco-friendly and less expensive without human health risks are preferred to control pathogenic fungi as compared to synthetic fungicides. Plant extracts or phytoextracts play a significant role as antifungal agents (Parveen et al., 2017; Haider et al., 2020).

Table 1. Test plants, English name or common name and traditional uses.

| Test plant name | Plant part used | English name/ Common name | Traditional uses |
|--------------------------------|-----------------|------------------------------|---|
| <i>Ocimum sanctum</i> | Leaves | Tulsi | The leaves are most popular for remedy of common cold, fever, bronchitis, arthritis, glaucoma, convulsions, dysentery, hemorrhage, dyspepsia, skin diseases etc. The leaves along with black pepper are used as a prophylactic against malaria (Prakash and Gupta, 2005). |
| <i>Solanum melongena</i> | Leaves | Bonbegun | Traditionally it has been used to treat tuberculosis, mouth ulcer, fever, hepatitis, stomach complaint, dysentery, dropsy, nausea, nervous disorders and other skin diseases (Nyeem et al., 2017). |
| <i>Bergera koenigii</i> | Leaves | Kari patta | The leaves are very famous for their effective treatment of inflammation, leukoderma, blood disorders, kidney pain and stop vomiting. The leaves are applied externally to cure skin eruption, relieve burns and treat the bite of poisonous animals. The boiled leaves with coconut oil are used as a hair tonic (Handral et al., 2012; Saini et al., 2015). |
| <i>Calotropis gigantea</i> | Leaves | Akondo | Used in the treatment of skin disorders, liver problems, wound healing, paralysis, rheumatic pains, stomach pain, and headache and are also used to treat migraine (Meena et al., 2011). |
| <i>Justicia adhatoda</i> | Leaves | Basak/Vasak | Leaves are primarily used for the treatment of cough, asthma, bronchitis, pneumonia, fever, jaundice, whooping cough common colds. The leaves juice is also applied to cure diarrhoea, dysentery and glandular tumor. It can be also used for the treatment of tuberculosis, intestinal worms and skin diseases (Dhankhar et al., 2011; Kharel, 2010). |
| <i>Andrographis paniculata</i> | Leaves | kalmegh | Used for the treatment of influenza, diabetes, high blood pressure, leprosy, flatulence, colic, ulcer, skin disease, bronchitis, malaria and skin diseases (Okhuarobo et al., 2014). |

2. Materials and Methods

2.1. Preparation of phyto-extracts

Fresh leaves from six selected medicinal plants viz, *Ocimum sanctum*, *Solanum melongena*, *Bergera koenigii*, *Calotropis gigantea*, *Justicia adhatoda*, *Andrographis paniculata* were collected from the fields of Singur college campus in Hooghly district. 10 g leaves samples were weighed and washed with sterilized distilled water followed by ethanol. For the preparation of ethanolic extract these samples were grinded in mortar and pestle separately along with mixing sand. Each paste was mixed with 100 ml ethanol to make a solution and kept in a closed container for 24 hrs at room temperature.

Like ethanolic extract, for the preparation of aqueous extract 10 g fresh leaves were washed with sterilized distilled water and then grinded in mortar and pestle. The paste sample was then mixed in 100 ml sterilized distilled water for each species and the solution become ready for test.

2.2. Test organism

The test organism *Curvularia lunata* was obtained from the earlier made pure culture in the laboratory and identified (Nagamani et al., 2006).

2.3. Method

The phytoextract solution (1 ml, 3 ml, 5 ml and 10 ml) of each species was separately poured on 15 ml autoclaved potato dextrose agar (PDA) medium on sterilized petri plates and allowed to be solidified. After solidification of the agar medium by the use of a cork borer (5mm) a punch of holes was done in the center of the agar medium in each petri plate and also in the pure culture of test organism. Then the fungal discs from the old culture were placed in the holes of the medium of all petriplates by cork borer under aseptic conditions. The petriplates were incubated at 27 ± 2 °C for 7 days (Ghante et al., 2019).

3. Results

A huge number of researchers in the world have studied the effect of phytoextracts against various microorganisms (Shekhawat and Vijayvergia, 2010). Results showed (Table 2; 3) six phytoextracts significantly inhibit the mycelial growth of the *Curvularia*. The ethanolic extract of *Bergera koenigii*, *Calotropis gigantea*, *Justicia adhatoda* and *Andrographis paniculata* shows the highest inhibition against test pathogen followed by *Solanum melongena* and *Ocimum sanctum*. The mycelial growth of pathogen against *Solanum melongena* and *Ocimum sanctum* (1 ml) was 1cm and 2 cm respectively which means *Solanum melongena* and *Ocimum sanctum* showed the least inhibition compare to other test phytoextracts. *Bergera koenigii*, *Calotropis gigantea*, *Justicia adhatoda* and *Andrographis paniculata* (1 ml, 3 ml, 5 ml and 10 ml) showed no mycelial growth of test pathogen. These ethanolic phytoextracts positively inhibit the growth of pathogens.



Figure 1. Pure culture of *Curvularia lunata*.

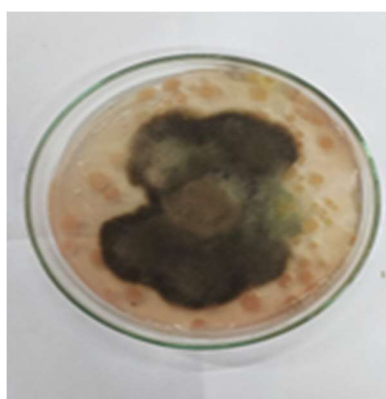


Figure 2. Control medium of *Curvularia lunata*.



Figure 3. Treatment with ethanol phyto extract of *Ocimum sanctum*.



Figure 4. Treatment with ethanol phyto extract of *Solanum melongena*.

Table 2. Antifungal activity of some medicinal plant ethanol extracts (100 ml) against *Curvularia lunata*.

| Plant species | Growth of fungi at different phytoextract solution | | | |
|--|--|------|------|-------|
| | 1 ml | 3 ml | 5 ml | 10 ml |
| <i>Ocimum sanctum</i> L. (Tulsi) | 2 cm | - | - | - |
| <i>Solanum melongena</i> L. (Bonbegun) | 1 cm | - | - | - |
| <i>Bergera koenigii</i> L. (Kari patta) | - | - | - | - |
| <i>Calotropis gigantea</i> (L.) W.T.Aiton (Akondo) | - | - | - | - |
| <i>Justicia adhatoda</i> L. (Basak) | - | - | - | - |
| <i>Andrographis paniculata</i> (Burm.f.) Wall. ex Nees (Kalmegh) | - | - | - | - |

“-” indicates no mycelial growth of the pathogen

Aqueous phytoextracts also play an important role to inhibit the growth of test pathogens like ethanolic phytoextracts. *Solanum melongena*, *Murraya koenigii* in all concentrations (1 ml, 3 ml, 5 ml and 10 ml) did not allow the growth of pathogen thus most effective plant extracts. The aqueous extracts of *Justicia adhatoda* showed zone of fungal growth were 1.5 cm (1 ml) and 1.3 cm (3 ml) and *Andrographis paniculata* showed 1.3 cm (1 ml) and 1.2 (3 ml). Both *Ocimum sanctum* and *Calotropis gigantea* showed fungal growth of 1cm (1ml) in diameter.

Table 3. Antifungal activity of some medicinal plant aqueous extracts (100 ml) against *Curvularia lunata*.

| Plant species | Growth of fungi at different phytoextract solution | | | |
|--|--|--------|------|-------|
| | 1 ml | 3 ml | 5 ml | 10 ml |
| <i>Ocimum sanctum</i> (Tulsi) | 1 cm | - | - | - |
| <i>Solanum melongena</i> (Bonbegun) | - | - | - | - |
| <i>Bergera koenigii</i> (Curry leaves) | - | - | - | - |
| <i>Calotropis gigantea</i> (Akondo) | 1 cm | - | - | - |
| <i>Justicia adhatoda</i> (Basak) | 1.5 cm | 1.3 cm | - | - |
| <i>Andrographis paniculata</i> (Kalmegh) | 1.3 cm | 1.2 cm | - | - |

“-” indicates no mycelial growth of the pathogen

The most effective aqueous plant extracts were *Solanum melongena*, *Bergera koenigii* followed by *Ocimum sanctum*, *Calotropis gigantea*, *Andrographis paniculata* and *Justicia adhatoda*. Therefore, the present study indicates that the antifungal activity is different in different plant species by the use of those materials.

4. Discussion

It was observed from the results that these plant extracts have significant inhibitory effects on the growth of *Curvularia*. The inhibiting efficacy of these phytoextracts against pathogenic fungi has been reported earlier. Parveen et al. (2017), Prakash and Gupta (2005) reported antifungal activity of *Ocimum sanctum* against *C. lunata*. It provides an important option as a biocontrol agent of *Curvularia*. Saini et al. (2015) showed the antifungal activity of the ethanolic extracts of *Bergera koenigii*. The antimicrobial properties of *Solanum* have been earlier reported by Nyeem et al. (2017), Sitap et al. (2015), Kotoky et al. (2011) and Kumar et al. (2016) reported the ethanol extracts of leaves of *Justicia adhatoda* showed in-vitro antifungal activity against pathogenic fungi. Recently Kumar et al. (2016), Sarkar et al. (2009), Karthikeyan et al. (2010) and Thokchom et al. (2011) studied the antimicrobial activities of *Adhatoda vasica*. Saratha and Subramanian (2010) and Goyal et al. (2013) have reported that *Calotropis gigantea* possesses a significant inhibitory effect on the test pathogen.

5. Conclusions

Many species of *Curvularia* reduce the growth and production of plants and cereals by causing diseases of them. They lose their natural quality and quantity. The primary objective of this study was to determine the inhibitory role of some medicinal plants viz. *Ocimum sanctum* L., *Solanum melongena* L., *Bergera koenigii* L., *Calotropis gigantea* (L.) W.T.Aiton, *Justicia adhatoda* L., *Andrographis paniculata* (Burm.f.) Wall. ex Nees against *Curvularia lunata*. Thus, the present investigation is an important step in developing eco-friendly pesticides as well as fungicides. Compared to other synthetic fungicide phytoextraction are very effective and safe for plant and human. It does not harm biodiversity. It can be easily used to control various plant pathogens.

Conflicts of Interests

Authors declare that there is no conflict of interests

Financial Disclosure

Author declare no financial support.

Statement contribution of the authors

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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