# In Vitro Evaluation of Antifungal Efficacy of Phytoextractsof Selected Plants against Early Leaf Blight of Tomato Causing Fungal Pathogen

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***Abstract:****Tomato (Lycopersiconesculentum Mill) belongs to family Solanaceae is being cultivated all over the world. Ripe fruits are used for the preparation of different items. It is thus most remunerable and widely grown vegetable in the world. Among the vegetables tomato ranks next to potato in world acerage and ranks first among the processing corps. However, the yield is severely affected due to different plant pathogens among which early leaf blight is one of them. In the present study, fungal pathogen was isolated from the infected leaves of tomato and fungicidal effect of different concentration of leaf extracts of – Azadirachtaindica, Nerium spp., Lantana camara, Partheniumhyterophorus, Ocimum sanctumand garlic cloves were determined. The isolated fungal pathogen was identified based on the macro and microscopic features. It was identified as Alternariasolani. Above phytoextracts were diluted for 20, 40, 60, 80 and 100% concentrations. It was noted that, 100% extract of garlic cloves gave the highest 88.20% inhibition of the mycelial growth of the above phytopathogenic fungus. At the same concentrations phytoextracts of Azadirachtaindica leaves inhibited radial growth of the fungus that was 86.79 and Ocimum sanctum leaf extract-85.96%. Here minimum inhibition of radial growth of the mycelium was 72.36% by the leaf extract of Partheniumhysterophorus. In the control the radial growth of the fungal pathogen was 89.0 mm. Above percentage was calculated on the basis of the radial growth of the fungus in the treated medium as well as in the control. Mancozeb, a common fungicide was used at 500 ppm to observe the fungicidal effect. Phytoextracts may be considered as a means of ecofriendly control measure of the diseases, there are no chances of contamination of soil or water, as is expected with the chemical fungicides, if phytoextracts are used to control the diseases of plants.*

***Keywords:*****In vitro, Lycopersicon Esculentum, Fungicides, Early Leaf Blight, Phytoextract, Mancozeb**

## 1. Introduction

Crops such as cereals, fruits in general and vegetables in particular are being damaged by different pathogens among which the fungal pathogens are more devasting. Leaf blight of tomato (*Lycopersiconesculentum*) is caused by *Alternariasolani*. In case of severe infection, maximum areas of leaves bear necrotic patches. They thus reduce the photosynthetic areas of the leaves and its impact is on growth of the plants and its yield. There are less fruit set and size of the fruits is also reduced. Even fruits are also infected that reduces the market value of the tomato fruits. To control these diseases different chemical fungicides are sprayed. Due to this the soils as well as local water bodies are contaminated. These fungicides if consumed through the food chain causes different kinds of health problems. Therefore, ecofriendly control measure is the best alternative. Among these phyto extracts are being used to control the fungal and bacterial pathogens. There are efforts to solve this challenging problem by the plant pathologists to search and promoted development of novel, ecofriendly and economical antifungal agents, which may replace the existing chemical fungicides that have long degradation periods, and therefore, are accumulating in our food chain.

Their accumulation in soil and water also damage useful microbes and insects in the ecosystem. From the survey of literatures we get several references where phyto extracts have been evaluated for their antifungal efficacy. Some of them are being mentioned here.

Singh and Majumdar (2001), evaluated efficacy of plant extracts against *Alternariaalternata* the causal agent of fruit rot of pomegranate. Onifade (2002) observed antifungal activity of extract of *Azadirachtaindica* against, *Colletotrichumlindemathianum*.Bajwa*et al*; (2004) studied antifungal activity of extracts of *Partheniumhysterophorus* against different fungal pathogens. Kiran and Raveesha (2006) observed antifungal activity of seed extracts of *Psoraleacorylifoia* L. Okigbo and Ogbonnaya (2006) observed antifungal effects of leaf extract of *Ocimumgratissiumum* and *Aframomummolegaeta* in case of post harvest yam rot. Davicino*et al*; (2007); Mohna and Raveesha (2007); Satish*et al*; (2007) all have evaluated extracts of different plants against different fungal pathogens. Badliya and Aikali (2008); Kumar *et al*; (2008); Mann *et al*; (2008) have observed the antifungal activity of crude extracts of different plants against different fungal pathogens. They found that these extracts have fungistatic property because they reduced the radial growth of fungal mycelium and sporulation.

Bobbaraha*et al*; (2009); Dubey*et al* (2009); Mandal*et al*; (2009); and Okegbo*et al*; (2009) all have evaluated extracts of different plants against different phytopathogenic fungi, for its fungicidal activities. Zaker and Mosallanejad (2010) observed antifungal activity of some plant extracts on *Alternariaalternata*, the causal agent of Alternaria spot of Potato. Ambikapathy*et al*; (2011); Dellavalle*et al*; (2011); Srivastava and Singh (2011); Tapwal*et al*; (2011) used extracts of different medicinal plants for the evaluation of antifungal activity against different phytopathogenic fungi. Bhardwaj (2012); Gujar and Talwankar (2012) and Ilondu (2012) studied the fungitoxic activity of extract of different plants. All concluded that extracts had positive results.

ChiejinanadUkeh (2013); Ganie*et al*; (2013); Jagtap*et al*; (2013); Sasode and Singh (2013); Sherwani*et al*; (2013); Singh and Srivastava (2013) evaluated antifungal activity of crude extract of different medicinal plants against different plant pathogenic fungi and observed that these extracts revealed antifungal activity. Ul-Haq*et al*; (2014) observed antifungal activity of extracts of certain medicinal plants against leaf spot disease of Mulberry (*Morus* spp.) Savaliya*et al*; (2015) evaluated phytoextracts against *Macrophominaphaseola* causing root rot of Sesame. Shazia*et al*; (2017) observed antimycotic activity of some phytoextracts on selected phytopathogenic fungi. Dar *et al*; (2018) reported *in vivo* impact of leaf extracts of certain medicinal plants through seed treatment and foliar spray against rice blast disease. Alverage*et al*; (2019) observed antifungal activity of extracts of mangrove against *Fusariumverticilloides* isolates. Keeping all the ideas in mind the present work was done to evaluate extracts of six different plants on mycelial growth of *Alternariasolani* isolated from the infected leaves of tomato plant.

## 2. Materials & Methods

Experiments were carried in the plant pathology laboratory of University Department of Botany, B.R.A. Bihar University, Muzaffarpur, Bihar. Infected tomato plants were located in the kitchen garden of local people of Muzaffarpur near the university campus.

### 2.1 Preparation of Culture Medium

*2.1.1 Potato Dextrose Agar Medium*

Healthy potatoes were purchased from the market. They were washed properly and peeled. 200 g of peeled and sliced potato was taken in 1 liter conical flask. To this 500 ml distilled water was added. It was boiled for 30-40 min and then filtered through muslin cloth. This potato infusion was taken in a graduated flask and the volume was made 1000 ml. To this 20 g Dextrose and 15 g Agar powder was added. Its pH was adjusted to 6.5. it was autoclaved at 15 lb pressure for 15-20 minutes. At this the temperature goes to $121^0$C. This was allowed to cool at $45^0$C. Before this to check bacterial growth streptomycin sulphate (3 grams) was added. Above medium was aseptically dispensed into pre-sterilized 9 cm diameter glass Petri plates. These plates were stored at low temperature for 3 days. Plates showing contamination were discarded after autoclaving.

*2.1.2 Isolation and Identification of Fungal Pathogen*

Leaves of tomato showing symptoms were collected from the kitchen garden where tomato plants were cultivated. These leaves were brought in the laboratory and were washed under running tap water for 30 minutes. These leaves were surface sterilized with 0.1% mercuric chloride for 3-5 minutes. They were washed with distilled water three times. During washing the flask containing the leaves was shaken vigorously for uniform contact of the surface. In this way even a trace of the chemical was removed to avoid toxic effect. These leaves were cut into small pieces in such a way that each piece had both healthy and infected portions. These small pieces were stored in pre-sterile and moist cloth. Inoculation was made in the aseptic chamber of Laminar flow. Inoculated plates were incubated at room temperature $27\pm1^0$C. After 7 days mycelial growth was seen. From this the subcultures were made in the PDA and incubated. The growth patterns and sporulation etc. were observed. The identification of isolated fungi was done macroscopically and microscopically. For microscopic study slides were prepared. For this small portion of the above cultures was taken and mounted in Lactophenol in cotton blue, on a clean glass slide. It was covered with clean cover slip and observed under microscope. The conidial structures, its septae, beaks etc. were observed and this was confirmed with the help of standard books of Mycology. The cultures were maintained in the laboratory for further experiments.

**2.2 Preparation of Extracts from the Selected Plants**

Plants, such as *Azadirachtaindica*, *Nerium*, *Lantana camara*, *Partheniumhyterophorus*, *Ocimum sanctum* were located in the university campus. The garlic bulbs were purchased from the local market.Fresh and healthy leaves of aforesaid plants were collected and brought in the laboratory. They were washed properly in the running tap water and then treated with 0.1% mercuric chloride for 3-5 minutes. All the leaves were rinsed in five changes of pre-sterilized distilled water. All the leaves were air dried at $28\pm2^0$C for one ot one and half hours. 20 grams, 40 gms, 60gms, 80 gms and 100 gms of each leaves of each plant were grounded in the pre-sterilized grinder in 100 ml distilled water. They were filtered through Whatman No.-1, filter paper separately into 250 ml conical flask. Thus 20%, 40%, 60%, 80% and 100% concentrations of extracts were obtained. Form the peeled garlic cloves, similar concentrations were obtained.

**2.3 Effect of Phytoextracts on the Percentage of Mycelial Growth of the Test Fungus**

PDA, medium was prepared. Now 1 ml of extract was mixed with 9 ml of molten medium, before it solidified. This was repeated for all the extracts separately. From the pre-cultures of the above fungus, 5 mm disc was taken from the periphery of 7 days old cultures. This was aseptically placed in the middle of the culture plates. Culture medium without extract was inoculated and used as control. Similarly, Mancozeb, 500 ppm was mixed in the medium and inoculated to observe the efficacy of the fungicide. All culture plates were incubated in culture room at $28\pm1^0$C. Radial growth of the fungal pathogen in treated Petri plates, in control and medium containing fungicide was measured after $7^{th}$ days of culture. Here the diameter of the mycelial disc was subtracted from the total length. Diameter was measured by the putting the plates upside down. Here tow lines, perpendicular to each other were drawn for the measurement.

The percentage inhibition of mycelial growth by each extracts, the fungicide was measured by using the formula of Vincent (1947).

$I = C\text{-}T / C \times 100$

Where,

I = Percentage of inhibition of mycelial growth.

C Mycelial growth of the fungus in control

T = Mycelial growth in the treated medium.

All the experiments were done in triplicate and each time 15 culture plates were used for each treatment. Mean of the data was placed in table-1 for results and discussion.

## 3. Result and Discussion

Based on the macroscopic symptoms, such as small lesions on the oldest leaves, presence of concentric rings, chlorotic regions around the leaf spots, and microscopic features of conidia such as 3-7 transverse septa and 2-4 longitudinal septa, short beak, and length and width the pathogen was confirmed

as *Alternariasolani* (Ellis and G. Martin). To evaluate antifungal activity of phytoextracts, six plants and five different concentrations of the extracts were separately supplemented in the PDA, culture medium at 1:9 ration. The mean of the data for antifungal activity was taken from the inhibition of radial growth of the test organism, the radial growth in control and in the presence of fungicide Mancozeb at 500 ppm were presented.

From the table it may be noted that in control the radial growth of the pathogen *Alternariasolani* was maximum that was 89 mm, whereas, in the plate containing 500 ppm of Mancozeb there was no growth at all. Among the phyto extracts, 100% extract of garlic cloves could inhibit 88.20 percent, which was followed by the same concentration of extracts taken from leaves of *Azadirachtaindica* which was 86.74%. This was followed by the leaf extract of *Ocimum sanctum*, 85.96%, *Lantana camara* 79.44% and *Nerium* spp. 75.73%. Here lowest percentage of inhibition of radial growth of *Alternariasolani* was 72.36, when the fungus was cultured in PDA medium supplemented with 100% leaf extract of *Partheniumhysterophorus*. It may be noted form the table-1, that all the phytoextracts, taken from the six different plants at their different concentrations revealed antifungal activity as indicated by the reduction in the radial growth, but the quantum of inhibition differed along with the concentration and the plants itself. Here extracts taken from garlic cloves at its all the concentrations, had higher rate of inhibition of radial growth, in comparison to the phytoextracts, taken from different plants. Next to this was the antifungal activity of extracts taken from leaves of *Azadirachtaindica*, where we get antifungal activity of phytoextracts at all the different concentrations.

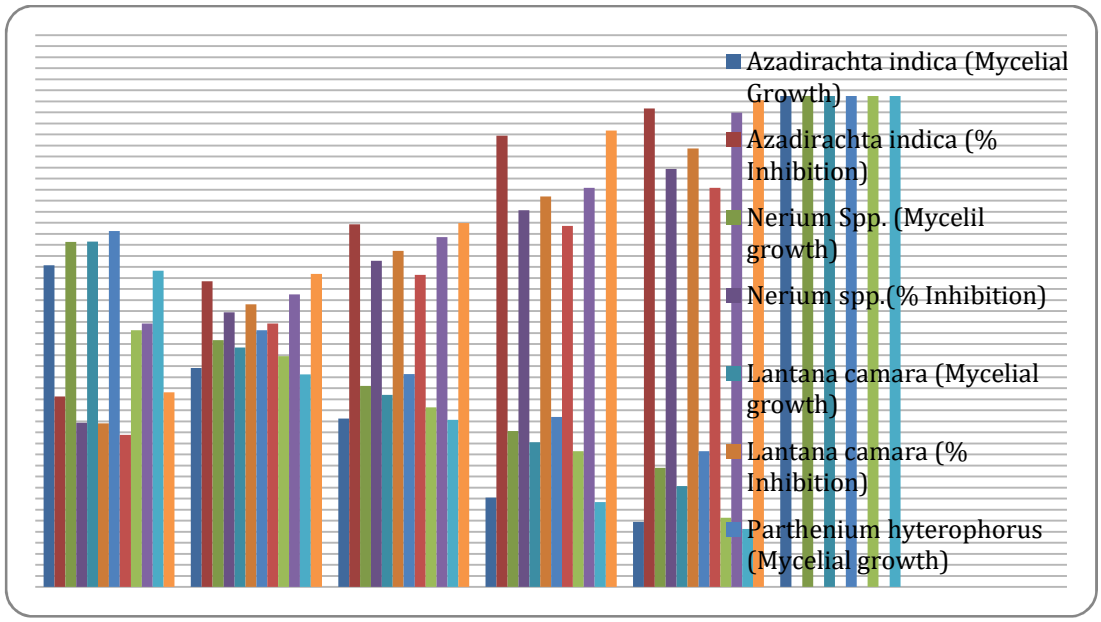
Antifungal activities of different phytoextracts have been evaluated by Dellavale*et al*; (2010). Who observed antifungal activity of medicinal plant extract against phytopathogenic fungus *Alternaria*Spp. They concluded that all the phytoextracts taken from nine different plants revealed antifungal activity but *Cynarascolymus*, *Salvia officinalis, Aloe vera* revealed maximum antifungal activity. The phyto extracts taken from*Lippiaalba* inhibited 98% mycelial growth of *Alternaria*. The percentage of inhibition increased along with the increase in the concentration of the extracts. In this way findings of the present work are in agreement with the above findings. Sherwani*et al*; (2013), concluded that antifungal activity of phyto-extracts are due to the presence of secondary metabolites such as flavonoids, alkaloids, saponins, anthroquinone, etc. They isolated above secondary metabolites from the leaf of *Carica papaya* and evaluated the phytoextracts for antifungal activity which was positive. Kantwa*et al*; (2014) reported i*n vitro* effect of fungicides and phyto extracts against *Alternariaalternata* causing leaf blight of ground nut. They used phyto extracts of 8 different plant species and concluded that different extracts inhibited the mycelial growth, but the percentage of inhibition increased along the increasing concentrations of the extracts. Findings of the present work therefore, corroborate with the above findings. Singh *et al*; (2014) also analysed different secondary metabolites of six different plants and concluded that the efficacy of phyto extracts as antifungal agent depended on the composition of secondary metabolites. Hussain*et al*; (2015) evaluated antifungal activity of some medicinal plants and conclude that extracts of *Azadirachtaindica* inhibited 95.93% of mycelial growth of *Aspergillusniger* followed by 90.74 by the extract taken from *Eucalyptus camaldulensis*. Ngegba*et al*; (2018) observed fungicidal effect of three plant extracts in control of four phytopathogenic fungi of tomato (*Lycopersiconesculentum* L.) fruit rot. They also observed that percentage of inhibition of mycelial growth depended on the concentration of the extracts used. Here also present findings are in agreement with the above findings. Singh *et al*; (2019) reported fungicidal effect of plant extracts on plant pathogenic fungi and the economy of extract preparation and efficacy in comparison to chemical fungicides. They also concluded that although the phytoextracts had antifungal activity at all the concentrations but the percentage of inhibition of mycelial growth increased along with the increase in the concentrations of the phyto extracts. It may be concluded that all the above findings indicate that antifungal activity of phytoextracts are related with the concentration of the extracts, as has been observed in the present work also.

## 4. Conclusion

Today yield of different crops in general and vegetables in particular are being influenced by different phyto pathogens among which fungal pathogens are most common. Usually we are using chemical

fungicides to control the fungal pathogens. But we are ignoring the damage being caused by the residual components of the chemical fungicides. There is demand for the ecofriendly control measures of these pathogens. Phytoextracts are the bestalternative of the chemical fungicides. Therefore, species specific control by the phytoextracts must be authenticated and popularized. So that our farmers can use them and thus one hand they will get cheaper device for the control on the other hand our ecosystem and food chain shall be free from the residual component of the fungicides.

**Graph 1:Showing fungicidal effects of different concentrations of Phytoextracts**




## Acknowledgements

The author is obliged to the Head, University Department of Botany, B.R. Ambedkar Bihar University, Muzaffarpur, for his kind permission to work in the departmental laboratory of plant pathology and avail library facilities.

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