



# Bio-efficacy of Ethanol Based Botanical Extracts against *Bipolaris maydis* Causing Maydis Leaf Blight in Maize (*Zea mays* L.)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Maydis leaf blight of maize caused by *Bipolaris maydis* has been observed as an alarming problem in maize growing regions leading to significant damage to crop and ultimately reducing grain yield production. This disease is most prevalent under humid and warm maize-growing areas worldwide. The alcoholic leaf extracts of four plants viz., *Ageratum conyzoides*, *Lantana camera*, *Eucalyptus*

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*globulus* and *Melia azedarach* were evaluated at different concentrations for their efficacy against *B. maydis* under *in vitro* conditions. Among all extracts, *Lantana camera* had maximum mycelial inhibition of 100 per cent followed by *Eucalyptus globulus* having 92.80 per cent of inhibition at 5 per cent test concentration, respectively. Thus, among all test botanicals, *Lantana camera* was found the most efficient against *B. maydis*. These findings suggest that *Lantana camera* extracts could be used as eco-friendly alternatives to control the maydis leaf blight of maize.

**Keywords:** *Bipolaris maydis*; maize; alcoholic botanicals; maydis leaf blight.

## 1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic situations. In India, maize is known as the “Queen of Cereals” because it has the highest genetic yield capacity among cereals and have many uses such as food (23%), feed (63%), starch industries (12%), seed and other purpose (2%) (Manzar et al. 2022). Two important amino acids i.e., lysine and tryptophan, are abundant in high-quality protein maize, which makes it crucial for improving the nutritional value of the daily diet (Malik et al. 2018; Haque et al. 2022). Maize is one of the primary crops of choice for industrial usage because of its versatility in agro-ecological and climatic conditions. In India, maize is the third-most important food crop after rice and wheat (Bharti et al. 2020).

In India, maize is cultivated over an area of 99,57,950 hectares, with a production 3,37,29,540 tonnes and productivity of 33,872 kg/ha from diverse soil types, climates, biodiversity and management practices (FAOSTAT, 2022a), whereas, In Himachal Pradesh, maize is mainly cultivated during *kharif* season under an area of 255.54 thousand hectares with a production of 708.42 thousand metric tonnes and a productivity of 2772.24 kg/ha (HPDOA, 2022b).

Amongst biotic stresses, Maydis Leaf Blight (MLB), caused by *Bipolaris maydis* (Nisik and Miyake) Shoemaker, (telomorph: *Cochliobolus heterostrophus*) is a serious fungal disease of maize throughout the world where maize is grown under humid and warm conditions (Kutawa et al. 2021). The disease is most common under warm, humid, temperate to tropical regions that have temperatures between 20 and 30°C throughout the cropping season (Bharti et al. 2017; Chandra et al. 2020; Vanlalhruaia et al. 2022). The disease has become well-known in India and has expanded throughout the plains, highlands and even certain areas of the peninsula (Kumar et al., 2022).

Frequent use of fungicides for disease management results in adverse effect and has led to various problems. The heavy reliance on chemical fertilizers can negatively impact soil health, raise environmental concerns, and diminish economic returns due to the high costs of inputs (Chen et al. 2022). Kursu et al. (2022) evaluated four ethanolic plant extracts against genus *Fusarium*, a major cereal pathogen. The extracts were used at concentrations of 5, 10 and 20 per cent. Out of four different ethanolic botanicals, the extract of sage (*Salvia officinalis* L.) and tansy (*Tanacetum vulgare* L.) demonstrated strong inhibitory effect of 83.53 per cent and 72.58 per cent against test pathogen at 20 per cent concentration, respectively. Pathak et al. (2020) evaluated various weed ethanolic extracts against Rhizoctonia Root rot (*Rhizoctonia solani*) in buckwheat and found that out of 4 ethanolic extracts evaluated, *Solanum nigrum* showed the highest inhibition (88%) at 500 ppm followed by *Tridax procumbens* (85%) and *Datura metel* (80%).

Ethanolic extracts alone or used in combination of fungicides can be proven an effective strategy for sustainable agricultural production systems. It can hold promise for developing biological, eco-friendly and non-hazardous antifungal products from various plant material. Hence, the present investigation was carried out to evaluate various ethanolic based botanical extracts against *Bipolaris maydis*.

## 2. MATERIALS AND METHODS

The present investigation was carried out in the laboratory at the Department of Plant Pathology, COA, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Himachal Pradesh (32.1000747N, 76.5468828E).

### 2.1 Isolation and Purification of the Pathogen

Under standard isolation protocol, leaves of maize plants exhibiting symptoms of Maydis leaf

blight were collected from maize-growing regions and isolation was done from infected samples on potato dextrose agar (PDA) medium. Inoculated plates were incubated for 7-10 days at  $27\pm1^{\circ}\text{C}$  to allow the fungal pathogen to grow. The pure colonies that developed from the bits were transferred to PDA slants and incubated at room temperature for 15 days. Pure culture of the fungus was maintained at  $4^{\circ}\text{C}$  for further studies.

## 2.2 Morpho-cultural Characterization of the Pathogen

The pathogen was identified on the basis of morpho-cultural characteristics. The isolated fungal pathogen was grouped based on colony morphology, colony colour, mycelial growth, shape and size of spores, spore septation and other characteristic features which was studied and recorded using microscope. Identifications were made after comparing the microscopic and morphological features of the pathogenic fungi with the available standard literature (Leonard and Suggs 1974; Navi et al. 1999).

## 2.3 In vitro Evaluation of Ethanolic Botanical Extracts Against *Bipolaris maydis*

### 2.3.1 Preparation of ethanol extract

Freshly harvested leaves of botanicals, namely, *Ageratum conyzoides*, *Lantana camera*, *Eucalyptus globulus* and *Melia azedarach* were thoroughly rinsed with tap water to remove impurities and were dried separately under the shade with occasional up and down mixing for 18-21 days. Subsequently, the dried leaves of each extract were finely powdered and kept in an airtight container. Ethanol was used as a solvent for the preparation of crude extract. 50 g powder of each leaf was soaked in 200 ml of solvent in 250 ml conical flask and it was kept on the rotary shaker at 150 rpm at room temperature for one day and then allowed to stand to settle the leaves debris. After the leaf debris settled on the bottom of the flask supernatant from each flask was filtered through Whatman No. 1 filter paper and evaporated at room temperature upto  $1/3^{\text{rd}}$  volume in warm water to completely remove the solvent. Concentrated air-dried extracts were then transferred into small vials and stored for further use at  $4^{\circ}\text{C}$ .

### 2.3.2 In vitro evaluation of ethanol extract

The antifungal effect of alcoholic-based extracts of four botanicals viz., *Ageratum conyzoides*,

*Lantana camera*, *Eucalyptus globulus* and *Melia azedarach* were evaluated *in vitro* at 5 concentrations (1, 2, 3, 4 and 5%) for their efficacy on inhibition in growth of *B. maydis* by Poisoned Food Technique (Falck, 1907). The experiment was conducted in a completely randomized design and each treatment was replicated thrice. The desired concentration of the crude extract was prepared by mixing it with the required quantity of sterilized PDA medium. Petri plates containing PDA medium without any amendments were served as control. After solidification of the medium, Seven-day-old mycelial bits of 5 mm of *B. maydis* were placed in the center of plates and incubated at  $27\pm1^{\circ}\text{C}$ . The radial growth of the fungal colony in each Petri plate was measured and per cent inhibition was calculated when control Petri plate was filled completely by using the formula given by Vincent (1947):

$$\text{Mycelial growth inhibition (\%)} = \{(C-T)/C\} \times 100$$

Where,

I = Per cent mycelial inhibition of test pathogen,  
C = Radial growth of pathogen (mm) in control  
T = Radial mycelial growth of pathogen (mm) in treatment

## 2.4 Statistical Analysis

The data of the experiment was pooled and subjected to appropriate statistical analysis. All the data were analysed in two-way analysis (ANOVA) using OPSTAT software. The significance of treatments was taken at 5 per cent level of significance. CD (Critical difference) is used to compare means of different treatments that have an equal number of replications.

## 3. RESULTS AND DISCUSSION

### 3.1 Identification of Pathogen

Isolation was made from disease samples of maydis leaf blight of maize collected from maize fields near the vicinity of Palampur. Based on morphological, cultural, and pathogenic characteristics, *Bipolaris maydis* was identified as the causal agent of maydis leaf blight of maize.

### 3.2 Morpho-cultural Characterization of the Pathogen

Microscopic examination of *Bipolaris maydis* revealed that mycelial was septate, branched

and olive greenish to brownish black. The conidiophores emerged singly or in small groups, which were straight or flexuous, septate and olive green to brown in colour. The conidia ranged from light to dark brown, varying in length from short to long and were straight or slightly curved, tapering towards rounded ends, widest near the middle, with up to 10 septate and smooth. Conidial production occurred at the tips and along the sides of the conidiophores. The length of conidia ranged from 69.32-79.51  $\mu\text{m}$  and width was 15.43-18.56  $\mu\text{m}$ .

Moderate growth of the *Bipolaris maydis* was observed on PDA medium. It attained 43.63 mm and 90 mm mycelial growth after four and nine days of incubation at  $27\pm 1^\circ\text{C}$  temperature. The texture of the colony was elevated; cottony growth with regular margins. The colony colour was initially white or whitish-grey, which later turned into olivaceous green-grey. Upon maturity it turned into dark black. The colony colour on underside of Petri plate was black. The shape of the colony was circular with smooth margins.

### 3.3 In vitro Evaluation of Ethanolic Extracts Against the Pathogen

The alcoholic leaf extracts of four botanicals viz., *Ageratum conyzoides*, *Lantana camera*, *Eucalyptus globulus* and *Melia azedarach* were evaluated at different concentrations i.e. 1, 2, 3, 4 and 5 per cent for their antifungal properties through Poisoned Food Technique against *B. maydis*. Radial growth and per cent mycelial inhibition was calculated when the control Petri

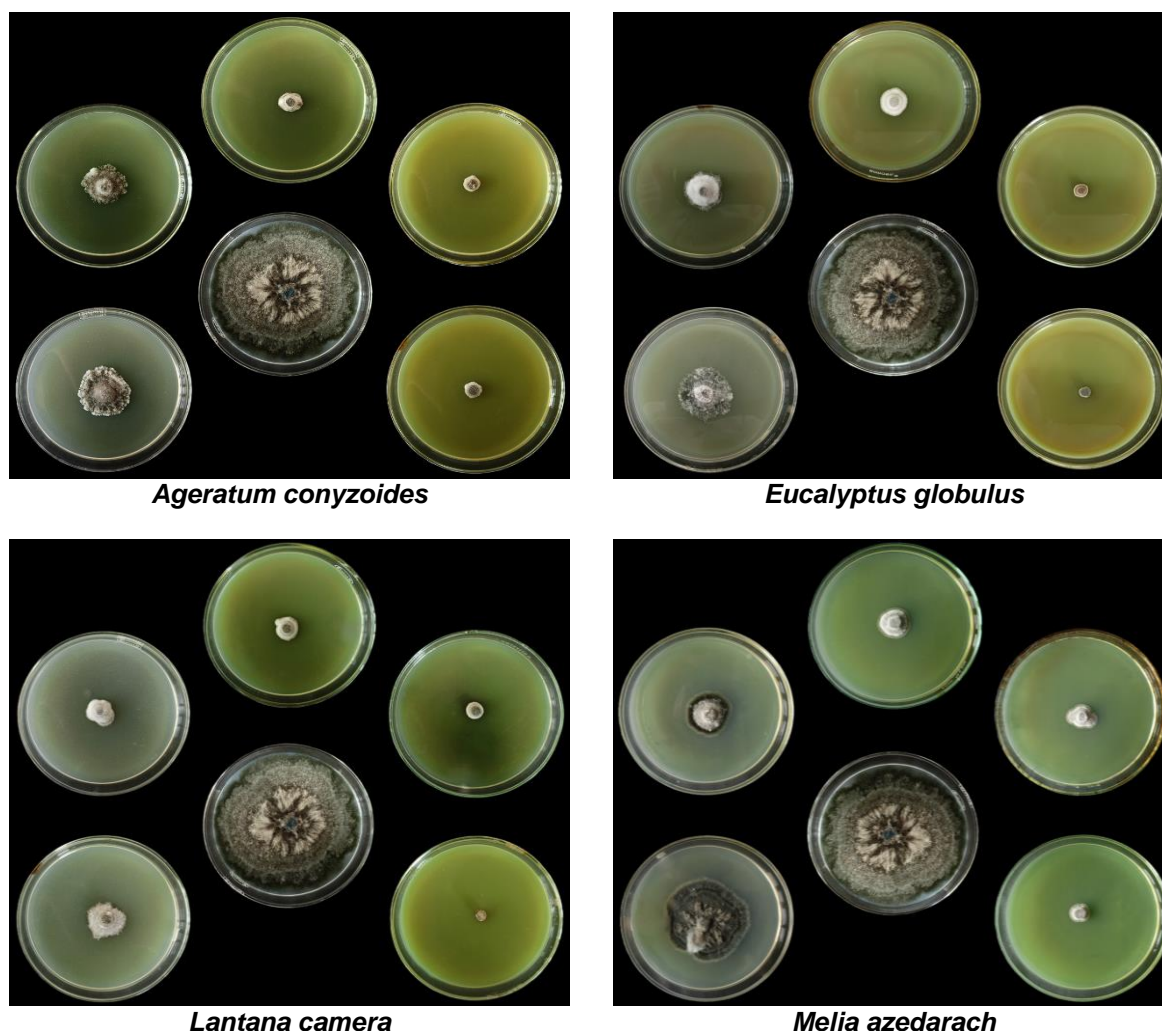
plate was completely filled (9 days) with mycelial growth of the pathogen. The data in the Table 1 revealed that alcoholic extracts of all the botanicals were found effective against the pathogen to a varying extent. Antifungal activities of all phytoextracts increased significantly with the increase in the concentration from 1 to 5 per cent. The alcoholic plant extracts of all botanicals proved to be effective against the pathogen at 5 per cent concentration resulted in more than 80 per cent mycelial inhibition. Among all four, *Lantana camera* was found best with mycelial inhibition of 100 per cent against *B. maydis* followed by *Eucalyptus globulus* (92.80%), *Ageratum conyzoides* (90.43%) and *Melia azedarach* (84.91%) at 5 per cent test concentration. The results obtained above have also been depicted in the bar diagram (Fig. 1). At 4 per cent concentration, both *Lantana camera* and *Eucalyptus globulus* shows per cent inhibition of 90.43 and 90.63 per cent, respectively, which are statistically at par with each other.

The results of these findings show the effectiveness of the extracts, the concentration-dependent response, and the relative performance of each botanical. All botanical extracts showed antifungal properties against *B. maydis* as indicated by the reduction in mycelial growth compared to the control (untreated). This reaffirms the potential of natural plant extracts as alternatives to synthetic fungicides in managing fungal pathogens. There was a noticeable trend of increasing antifungal activity with higher concentrations of the extracts.

**Table 1. In vitro efficacy of alcoholic extract of botanicals against *Bipolaris maydis***

Botanical	Radial growth (mm)					Inhibition in mycelial growth (%)				
	Concentration (%)					Concentration (%)				
	1	2	3	4	5	1	2	3	4	5
<i>Ageratum conyzoides</i>	31.69 (5.71)*	23.46 (4.94)	15.47 (4.05)	11.52 (3.53)	8.61 (3.09)	64.79	73.94	82.81	87.20	90.43
<i>Lantana camera</i>	22.40 (4.83)	18.56 (4.42)	13.54 (3.81)	8.61 (3.09)	0.00 (1.00)	75.11	79.38	84.96	90.43	100.0
<i>Eucalyptus globulus</i>	30.57 (5.61)	22.35 (4.83)	18.58 (4.42)	8.44 (3.07)	6.48 (2.72)	66.03	75.16	79.36	90.63	92.80
<i>Melia azedarach</i>	55.56 (7.52)	30.50 (5.61)	23.70 (4.96)	20.55 (4.64)	13.58 (3.81)	38.26	66.11	73.67	77.16	84.91
Control	90.00 (9.53)	90.00	90.00	90.00	90.00 (9.53)	0.00	0.00	0.00	0.00	0.00
	CD ( $p=0.05$ )									
Botanicals (A)	0.078									
Concentrations (B)	0.078									
Factor (AxB)	0.174									

\* Figures in the parentheses are angular transformed values

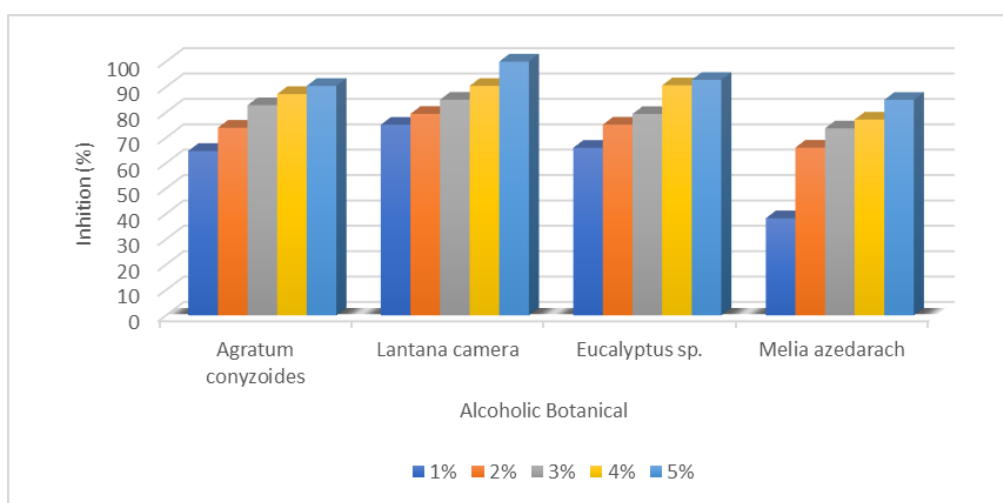


**Fig. 1. Mycelial inhibition of *Bipolaris maydis* with alcoholic extract of botanicals after nine days**

This concentration-dependent response suggests a dose-response relationship, where higher concentrations of the extracts led to greater inhibition of fungal growth. This aligns with the principle that higher concentrations of bioactive compounds present in the extracts can exert stronger inhibitory effects on the pathogen. So, among the four botanicals tested, *Lantana camera* emerged as the most effective, demonstrating the highest mycelial inhibition of 100 per cent at the 5 per cent concentration. This suggests that *Lantana camera* possesses potent antifungal compounds that are particularly effective against *B. maydis*. The antifungal potential of aqueous and crude extracts of botanicals against pathogens have also been reported by various workers in the recent past.

Rehena et al. (2022) evaluated eight ethanolic plant extracts for controlling wheat blast caused

by *Magnaporthe oryzae* and recorded that the lowest mycelial growth was recorded with Aloe vera extracts and *Nigella sativa* (black cummin seeds) extracts at 1:1 w/v and 1:0,25 w/v, respectively at 7 days after inoculation. Chhabra and Sharma (2023) evaluated two botanicals with two different solvents i.e., aqueous and 50% ethanol against sheath blight of rice and recorded that ethanolic extracts have higher number of secondary metabolites than aqueous one. Kaur et al. 2024 test botanicals, under which *Eupatorium adenophorum* was found the most efficient against *F. solani* f. sp. *pisi*. These results highlight the potential of these botanical extracts as natural antifungal agents for managing *B. maydis*. However, further studies are required to elucidate the specific bioactive compounds responsible for the observed antifungal activity and to assess their efficacy under field conditions.



**Fig. 2. Mycelial inhibition (%) of the different treatments at varying concentrations on the growth of *Bipolaris maydis***

#### 4. CONCLUSION

The present investigation highlighted the potentialities of ethanolic leaf extracts in managing Maydis leaf blight of maize. Increasing concentrations of the extracts correlated with higher efficacy, having *Lantana camera* exhibiting the most significant inhibition of mycelial growth followed by *Eucalyptus globulus*. The management of Maydis leaf blight in maize through ethanolic extracts may offers a sustainable and environmentally responsible alternative to conventional chemical control methods. Due to increasing regulatory and consumer-driven demand for sustainable agriculture, the integration of ethanolic based plant extracts may act as a viable long-term strategy for effective MLB and other fungal diseases management in agriculture.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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