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## Effect of some bio-products on the growth of *Helminthosporium maydis*, causal agent of Southern Corn Leaf Blight by dual culture technique under *in-vitro* condition

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### Abstract

Maize belongs to the family Poaceae, is the third most important cereal crop under irrigated and rainfed agricultural system in semi-arid and arid tropics and also known as queen of cereals. The yield of this crop is challenged by a number of biotic stresses among which maydis leaf blight disease caused by *Helminthosporium maydis* / *Bipolaris maydis* is very much important. Proper management of this disease should be followed for minimizing the loss due to a disease attack. Cultural, chemical and biological control measures are important ways of disease management. Control of plant diseases by using biocontrol agents and natural / plant products is an eco-friendly & sustainable approach. Considering the hazardous effects of fungicides and chemicals, in this experiment for the purpose of management of MLB of maize by using eco-friendly ways three bio-control agents and three natural products were tested against *H. maydis* under *in-vitro* condition. Two plant extracts *Allium Sativum* (garlic), *Azadirachta indica* (neem), three bioagents *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescence* and one natural product cow urine were tested against the growth of *Helminthosporium maydis* under *in-vitro* condition. Among the six treatments minimum radial growth (1.13cm) & maximum growth inhibition (82.8%) of *Bipolaris maydis* was found in case of application of *Trichoderma harzianum* followed by *Trichoderma viride* (radial growth – 1.30cm & growth inhibition 80.21%) with no significant difference among them. The growth inhibition of 35.61% was recorded in case of *Pseudomonas fluorescens* respectively. Better result was obtained when three bio-control agents were applied but among the natural products better result (radial growth 4.47cm & growth inhibition 31.96%) was obtained with application of garlic bulb extract (10%) followed by application of neem extract (25.11). Six botanicals were evaluated *in-vitro* against *Helminthosporium maydis*. The result revealed that, bulb extract of garlic (*Allium sativum*) at 10 per cent concentration completely inhibited mycelial growth of the fungus. Among the six treatments minimum radial growth (1.13cm) & maximum growth inhibition (82.8%) of *Bipolaris maydis* was found in case of application of *Trichoderma harzianum* followed by application of *Trichoderma viride* (radial growth – 1.30 cm & growth inhibition 80.21%) with no significant difference among them. Better result was obtained when three bio-control agents were applied but among the natural products better result (radial growth 4.47cm & growth inhibition 31.96%) was obtained when garlic bulb extract (10%) was applied.

**Keywords:** Maydis leaf blight, bioagent, natural products, radial growth, growth inhibition

### Introduction

Maize (*Zea mays* L); Zea means “Sustaining life” and mays meaning “Life giver”. It is the third most important cereal crop of India after Rice & Wheat. Known as “Queen of Cereals” because of its highest yield potential. Thermo & Photo insensitive nature of this cereal crop allows it to grow throughout the year. Yield of maize is affected by a number of biotic stresses. Southern Corn Leaf Blight caused by *Bipolaris maydis* (Ascomycetes) is one of them. It is called southern leaf blight (SCLB) because it was widespread in Southern United State. The epidemic in USA due to SCLB was very much severe and this is an example of huge economic loss by a plant disease (Strobel, 1975) [7]. It is a severe problem of maize specially in those pockets having warm and humid weather conditions (White, 1999) [10]. Upto 70% yield loss is recorded due to SCLB (Wang *et al.* 2001; Ali *et al.* 2011) [9, 11]. Among three races (race O, race T & race C) of *Bipolaris maydis* (Smith *et al.*, 1970; Wei *et al.* 1988) [6, 11] in India race O is the

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prominent one. In India first reported from Maldah district of West Bengal by Munjal & Kapoor in 1960<sup>[4]</sup>. Characteristic symptoms are – initially small diamond shaped spots, become elongated with maturity, coalesce to produce complete burning of large areas of leaves. This disease can be managed by using resistant varieties and chemicals. Chemicals are not safe if environmental safety is considered and should be avoided. To evaluate the efficacy of some bio-control agents and natural products against SCLB for avoiding the hazardous effect, environmental pollution & residual toxicity of chemicals in management of this disease.

### Materials & Methods

Two plant products and one natural product were tested against *Bipolaris maydis* 10 grams of fresh leaves /plant parts were collected, washed & ground using pestle and mortar by adding equal amount (10 ml) of sterilized distilled water (1:1w/v). The extract was filtered through muslin cloth and centrifuged at 2000 rpm for 30 minutes at ambient temperature (26 ±2°C). This supernatant was used as standard plant extract solution (100%). The plant extracts were tested at 10 per cent concentration and this was achieved by incorporating 10 ml supernatant of each plant extract into 90 ml of PDA media in conical flasks respectively and sterilized by autoclaving at 15 psi for 20 minutes. 20 ml of PDA containing plant extract was poured into sterilized petriplate under aseptic conditions and allowed to solidify. PDA plates containing the plant extracts were inoculated aseptically in the center with 5 mm disc of ten days old young sporulating culture of *Helminthosporium maydis*. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment. The inoculated petriplates were incubated at 27±1°C in the laboratory. The colony diameters were measured when the fungus touched the periphery in control plates.

Three bioagents were tested against *Bipolaris maydis*. 10ml of PDA was poured into sterile petriplates and allowed to solidify. Previously grown young cultures of both fungal bio

agents and host pathogen 5 mm disc of the test fungus and respective bio agents were transferred aseptically to petriplates simultaneously by leaving sufficient space in between two discs. Three replications were maintained for each treatment. The petriplates were incubated at 28±1°C till the growth of colony touches the periphery in the control plate. Colony diameter of both the test pathogen and bio agents were measured.

**Table 1:** *In vitro* evaluation of six treatments

S. No	Treatment	Naturals, & Bio agent
1	T <sub>1</sub>	<i>Trichoderma viride</i>
2	T <sub>2</sub>	<i>Trichoderma harzianum</i>
3	T <sub>3</sub>	<i>Pseudomonas fluorescens</i>
4	T <sub>4</sub>	Garlic bulb extract
5	T <sub>5</sub>	Neem leaf extract
6	T <sub>1</sub>	Cow urine

Per cent inhibition of growth was calculated by using formula given by Vincent (1947)<sup>[8]</sup>.

$$I = \frac{C-T}{C} \times 100$$

Where, I = Percent inhibition, C =Colony diameter in control (cm), T = Colony diameter in treatment (cm)

### Result & Discussion

Proper management should be followed for minimizing the loss due to a disease attack. Cultural, chemical and biological control measures are important ways of disease management. Control of plant diseases by using bio-control agents and natural/plant products is an eco-friendly & sustainable approach. For the purpose of management of MLB of maize by using eco-friendly ways three bio-control agents and three natural products were tested against *H. maydis* under *in-vitro* condition.

**Table 2:** Effect of some bio-products product on the growth of *Helminthosporium maydis* under dual culture technique under *in vitro* condition.

Treatment	Radial growth of pathogen(cm)*	*Growth inhibition (%)
<i>Trichoderma harzianum</i>	1.13	82.8
<i>Trichoderma viride</i>	1.30	80.21
<i>Pseudomonas fluorescens</i>	4.23	35.61
Garlic bulb extract	4.47	31.96
Neem extract	4.92	25.11
Cow urine	5.20	20.73
Control	6.57	---
C.D. at 5% level	0.227	3.41
SE (m)	0.073	1.095

\*Average of three replication

Among the six treatment minimum radial growth (1.13cm) & maximum growth inhibition (82.8%) of *Bipolaris maydis* was found in case of application of *Trichoderma harzianum* (plate-2) followed by *Trichoderma viride* (radial growth – 1.30cm & growth inhibition 80.21%) with no significant difference among them which result was also found by Kumar *et al.* (2009)<sup>[3]</sup> the efficacy of three bio agent's viz. *Trichoderma harzianum* and *Trichoderma virens* against *Helminthosporium maydis*. *T. viride* was found most effective inhibiting the mycelia growth of fungus. The growth inhibition of 35.61% was recorded in case of *Pseudomonas fluorescens* respectively.

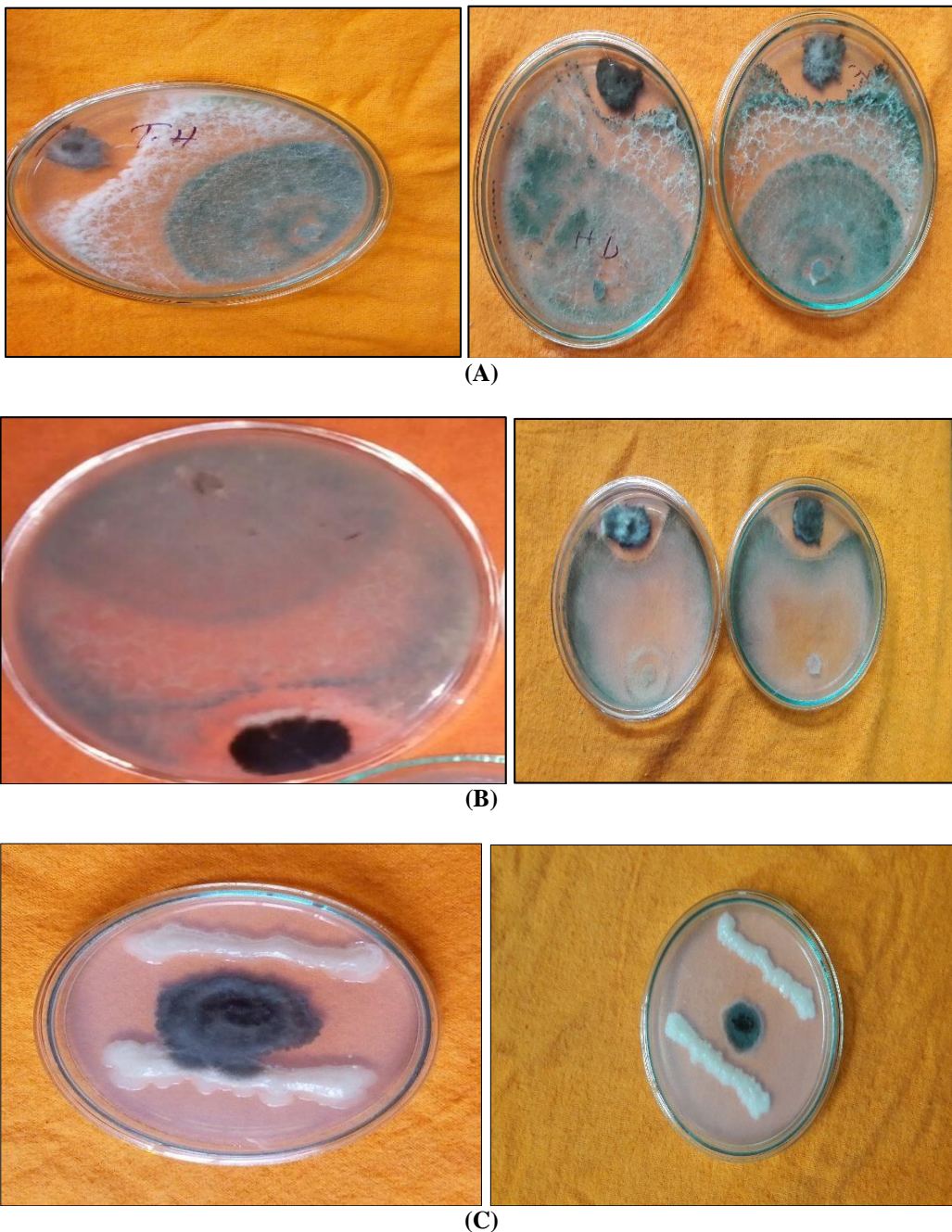
Better result was obtained when three bio-control agents were

applied but among the natural products better result (radial growth 4.47cm & growth inhibition 31.96%) was obtained when garlic bulb extract (10%) was most effective followed by neem extract (25.11%) applied which is very much similar to the opinion of Sanjeev Kumar *et al.* (2009)<sup>[3]</sup> - garlic extract was highly effective in inhibiting the growth of *Helminthosporium maydis* as it produced 66.5, 73.8 and 83.9 per cent growth inhibition at 2, 5 and 10% concentrations respectively and that of Khamari *et al.*, (2015)<sup>[2]</sup> - maximum inhibition of the fungus mycelium growth *Helminthosporium maydis* from *Allium sativum* with 72.6 % and 100% at 10 % and 20 % concentrations among all plant extracts with same observation of Kumar *et al.* (2009)<sup>[3]</sup> - six botanicals were



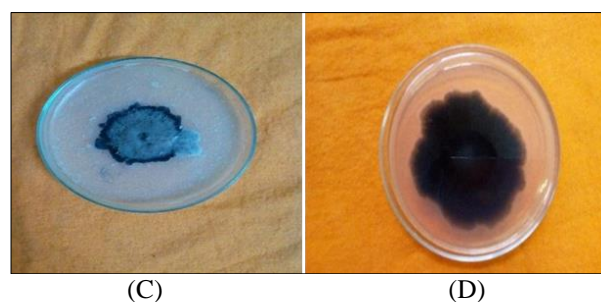
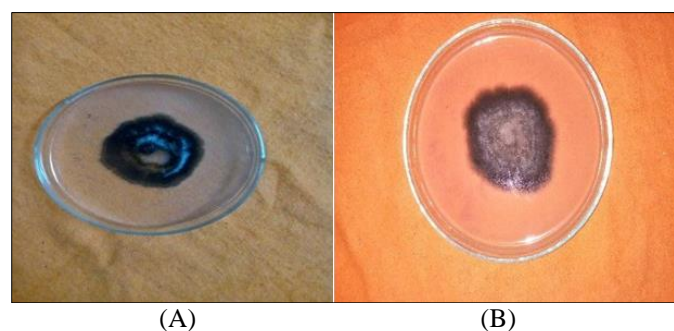
evaluated *in vitro* against *H. maydis*. The result revealed that, bulb extract of garlic (*Allium sativum*) at 10 per cent

concentration completely inhibited mycelial growth of the fungus.



**Fig A:** Growth Inhibition by *Trichoderma Harzianum*  
**B:** Growth Inhibition by *Trichoderma Viride*  
**C:** Growth Inhibition by *Pseudomonas Fluorescens*

**Growth inhibition of *Helminthosporium maydis* by bioproducts**



**Fig A:** Growth Inhibition by Neem Extract.  
**B:** Growth Inhibition by Cow Urine.  
**C:** Growth Inhibition by Garlic Extract.  
**D:** Growth on Control

## Conclusion

Minimum radial growth & maximum growth inhibition of the fungus *Bipolaris maydis* was found after application of *Trichoderma harzianum* followed by *Trichoderma viride*. Better result was obtained when three bio-control agents were applied but among the natural products comparatively better result was obtained when garlic bulb extract was applied.

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