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Efficacy of bio-agents, botanical extracts and essential oils against southern leaf blight of maize (*Zea mays* L.)

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Abstract
An experiment was conducted to evaluate the effect of plant extracts, bio agents, essential oils and fungicide in vitro and *In vivo* against *Helminthosporium maydis* causing Southern leaf blight of maize. Mancozeb @ 0.2% was effective in the inhibition of mycelial growth (100%) of *Helminthosporium maydis*. Among the essential oils, Lemongrass oil @ 0.2 %was found effective in the inhibition of mycelial growth (100%) followed by neem oil @ 0.1% (57.58%). Among the bio-agents, *Trichoderma viride* was found effective in the inhibition of mycelial growth (78.89%). Among the plant extracts, *lantana camara* leaf extract @ 15% was found effective in the inhibition of mycelial growth (70.97%) followed by onion bulb extract @ 10% (57.58%) and neem leaf extract @ 10% (48.13%). The plant extracts, potential bio-agent and fungicide found effective *in vitro* were tested and the effect of selected treatment on southern leaf blight disease intensity under field conditions was assessed during Rabi season 2018-2019. Among all the treatments, the maximum yield of maize was recorded in Mancozeb @ 0.2% (41.66q/ha) followed by *Trichoderma viride* (39.16 q/ha), Lemon grass oil @0.2% (37.98 q/ha), T5-Neem oil @ 0.1% (34.33 q/ha), *Lantana camara* @15% (32.42 q/ha), Neem leaf extract @10%(30.83 q/ha), and Onion bulb @ 10 % (30.50 q/ha) as compared to the untreated control(25.00 q/ha).

Keywords: Southern leaf blight of maize, maydis leaf blight, *Helminthosporium maydis*, *Trichoderma viride*, lemon grass oil, neem oil

Introduction
Maize is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Purseglove, 1992) [10]. Among the several diseases of maize, southern leaf blight is one of the economically significant disease adversely affecting the vegetative plant parts and seeds and in turn the production. Southern Corn Leaf Blight (SCLB) or Maydis Leaf Blight (MLB) caused by *Helminthosporium maydis* (Syn. *Bipolaris maydis* (Nisik.) Shoemaker), (telomorph: *Cochliobolus heterostrophus*) is a serious fungal disease of maize throughout the world where maize is grown under warm, humid conditions (White, 1999). Dreschler (1925) [14, 3] Southern leaf blight is one of the common and destructive diseases of Maize in India. Bugnicourt (1955) [2] first studied *Helminthosporium sp*. and described morphological character as colonies were effuse, grey to blackish brown or grey, stromata sometimes formed in culture, erect, straight cylindrical and black hyphae of *Helminthosporium* were pale to mild brown, smooth, septate and about 1-3 µm thick. The conidia were straight, ellipsoidal, oblong or cylindrical, round at the ends, pale to mid brown. The disease is characterized by the loss of photosynthetic leaf area, due to foliar lesions which reduce photosynthetic production for grain filling. Further damage is caused by lodging, which occurs when plants divert sugars from the stalks for grain filling during severe disease pressure. Damage is most critical if infection occurs prior to silking and if weather conditions are favourable for disease development during the reproductive growth stages. Seedlings grown from kernels infected with the pathogen may die four weeks following planting. There are several important reports on the biological management of this disease. The losses due to southern leaf blight can be managed through the foliar application of chemical fungicides. Utilization of plant extracts, bio agents and essential oils in disease management is considered as eco-friendly, without any environmental pollution. The present study was carried out to explore, the efficiency of some plant extracts and *Trichoderma spp*. against southern leaf blight of maize caused by *Helminthosporium maydis In vitro* and *in vivo* at Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, (U. P), India.

Materials and Methods

Isolation of the pathogen

The fungus was isolated by tissue isolation technique. The diseased tissues along with some healthy leaf portion (1-2 mm^2) were cut with a sterilized razor blade at the margins of the diseased spots and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds. The segments were then rinsed thrice in sterilized distilled water to remove the traces of mercuric chloride solution, blotted dry and placed on acidified potato dextrose agar (PDA) medium (pH adjusted to 6.5 with N/10 HCl) in sterilized petri plates under aseptic conditions. The inoculated PDA plates were incubated at 26±1 °C for 7 days and sub cultured onto fresh PDA medium at the same temperature for 15 days. Examination of the fungal colony characteristics were done through microscopic examination. Using a sterile needle, a small portion of the culture is taken and placed on a sterile glass slide. It is stained using lacto phenol and cotton blue. Then, the microscope is used for the examination of morphology and culture characteristics of fungal structures. Conidia appeared elongate, cylindrical to slightly curved, smooth and mostly rounded but tapering toward both ends. Conidia were phragmospore showing 6-10 septa. Conidia revealed 67 to 155ìm × 21 ìm size (Bhavani *et al.*, 2016) [1].

Purification by single spore technique

Helminthosporium maydis being a sporulating fungus was purified by single spore technique (Johnston and Booth, 1983) [6]. Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing 8 to 10 spores per microscopic field at low power from 15 days old culture. One ml of such suspension was spread uniformly on 2 per cent solidified water agar plates and incubated at 25±1 ºC for 12 hours. The plates were examined under stereoscopic microscope; single spores were marked with a marker, allowed to germinate and finally picked up using cork borer and transferred aseptically to potato dextrose agar medium in sterilized petri plates for further growth in the incubator at 25±1 ºC. The pure culture thus obtained, was used for further studies.

In vitro Experiment

Aqueous extract of plant parts such as leaves, bulbs were prepared by using the standard method as given by Gerard Ezhilan *et al.* (1994) [4] Essential oils collected from Department of Plant Pathology. The plant extract thus prepared and neem oil and lemon grass oil were tested against *Helminthosporium maydis* using poisoned food technique. The plant extracts, Neem leaf extract (10%), garlic bulb extract (10%), lantana leaf extract (15%) and one chemical Mancozeb (0.2%) and lemon grass oil (0.2%), Neem oil (0.1%) were tested for their efficacy against the pathogen *Helminthosporium maydis In vitro* using poison food technique on Potato dextrose agar medium.

The calculated concentrations of fungicides were thoroughly mixed in the medium. Twenty ml of amended medium was poured in 90 mm sterilized petri dishes and allowed to solidify. Mycelial disc of 5 mm from seven day old actively growing culture was inoculated at the centre of the petri plate and then incubated at 28 ± 2 °C for 7 days. Control was maintained without any treatment. Three replications were maintained for each treatment and data were recorded at 96 hours after inoculation. The antagonistic microorganisms, *Trichoderma viride* was tested in vitro for its antagonism against *Helminthosporium maydis* by dual culture technique. Twenty millilitre of PDA was poured into sterile petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3 to 4 cm gap. One control was maintained where in only test fungus was grown. The plates were incubated at 28 ± 2 °C for 96 hours. The experiment was conducted in completely randomized block design (RBD). Per cent inhibition of mycelial growth calculated using the following formula (Vincent 1927) [13].

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

Where,
I = Per cent inhibition of mycelial growth
C = Colony diameter in control (mm)
T = Colony diameter treatment (mm)

In vivo Experiment

In order to check effect of foliar spray of fungicide, plant extracts and bio-agents on Southern leaf blight of maize under field condition, field experiments was laid-out in Randomized block design with three replications. Two sprays were given at an interval of 30 days. Treatments were imposed after appearance of the first disease symptoms. Observations on disease severity of *Helminthosporium maydis* were recorded by using 1-5 disease rating scale of (Payak and Sharma 1982) [9] at 30 days interval and yield data were obtained after the harvest on physiological maturity.

Results and Discussion

The Plant extracts like *Azadirachta indica* (10%), *Allium cepa* (10%), *Lantana camara* (15%), lemongrass oil (0.2%) and Neem oil (0.1%) were tested against *Helminthosporium maydis*. All the botanicals and essential oils tested were significantly effective in inhibiting the growth of pathogen over control (Table 1). Among tested treatments by poison food technique, Lemon grass oil at 0.2 per cent showed maximum inhibition (100%) followed by neem oil at 0.1 per cent (72.52%) and least effectiveness was found in Neem leaf extract (48.13%).

Table 1: Mycelial growth (mm) of *Helminthosporium maydis* affectted by Plant extracts (Poison food technique)

S. No	Treatments	Radial growth (mm) of *Helminthosporium maydis*
		Average mean at 9th day
T7	Mancozeb	0
T6	Lemon grass oil	0
T5	Neem oil	20.83
T4	*Lantana camara*	22.00
T3	Onion bulb	32.16
T2	Neem	39.33
T0	Control (untreated check)	75.83
	S.Ed (±)	0.6785
	CD (0.05%)	1.4587

**Average mean of three replications

The fungus *Trichoderma viride* bio-agent was evaluated in vitro against *Helminthosporium maydis* by dual culture technique and using Potato dextrose agar as basal medium. From the *in vitro* evaluation of *Trichoderma viride*, the observations revealed that the maximum reduction in colony growth of was recorded (78.89%). The results (Table 2) revealed that *Trichoderma viride* exhibited fungi static activity and significantly inhibited mycelial growth of *Helminthosporium maydis*. Similar findings were reported by Kumar *et al.* (2009) [11] in their studies they found that among all the antagonists, *Trichoderma viride* showed the highest inhibition of radial growth of *Helminthosporium maydis.*

Table 2: Mycelial growth (mm) of *Helminthosporium maydis* affected by bio -agents (Dual culture technique)

S. No	Treatments	Radial growth (mm) of *Helminthosporium maydis*
		Average mean at 9th day
T1	*Trichoderma viride*	16.00
T0	Control (untreated check)	75.83
S.Ed (±)		0.928
CD (0.05%)		2.579

**Average mean of three replication

A field study was carried out to assess the efficacy of *Trichoderma sp*, Plant extracts, essential oils and fungicide against southern leaf blight (*Helminthosporium maydis*) of maize with two sprays taken up at 30 DAT and 60 DAT during Rabi season 2018-2019 (Table 3). Minimum disease intensity was recorded in *Trichoderma viride* at 30, 60 and 90 days after spray (10.90%, 22.00% and 39.40% respectively) followed by, Lemon grass oil (11.79%, 22.89%, 40.45% respectively), Neem oil (11.79%, 23.00% and 42.16% respectively), *Lantana camara* (12.55%, 23.76% and 43.55% respectively) and treated check Mancozeb showing disease intensity (8.79%, 19.89% and 38.44% respectively) was also found effective and found statistically significant from control (14.68%, 26.25% and 46.84%, respectively). Earlier the minimum disease intensity in case of MLB have been recorded in case of crop sprayed with *Trichoderma viride* by Kumar *et al.* (2015) [5] and Malik *et al.* (2018) [7]. Followed by *Trichoderma viride* essential oils like Lemon grass oil and Neem oil found to be effective in controlling the disease. Similar findings have been reported by Santos *et al*. (2013) [12], who revealed that amongst the four essential oils evaluated lemon grass oil was found to be best in inhibiting the mycelial growth of the pathogen when compared with other essential oils.

Table 3: Effect of treatments on disease intensity at 30, 60 and 90 DAT

Treatment No.	Treatments Name (Foliar spray)	Disease Intensity At (90 DAS)	Yield at maturity
T0	Control	46.84	25
T1	*Trichoderma viride*@ 0.5%FS	39.40	39.16
T2	Neem Leaf extract 10% FS	46.08	30.83
T3	Onion Bulb @10% FS	46.10	30.50
T4	*Lantana Camara* @15% FS	43.55	32.42
T5	Neem oil @0.1% FS	42.16	34.33
T6	Lemon grass oil @0.2% FS	40.45	37.98
T7	M-45 @ 0.2%FS	38.44	41.66
S. Ed. (±)		0.681	1.126
C.D. (P = 0.5)		1.445	2.446

Conclusion

The present study conducted Efficacy of Bio agents, Botanical extracts and Essential Oils against *Helminthosporium maydis* causative agent of Southern leaf blight of Maize. From the critical analysis of the present finding it was concluded that by observing all these treatments it was found that lemon grass oil and *Trichoderma viride* are most effective in inhibiting the disease both in *in vitro* and *in vivo* which are eco-friendly to the environment. The maximum yield of maize was recorded in *Trichoderma viride* (39.16q/ ha), Lemon grass oil (37.98q/ha), Neem oil (34.33q/ ha) as compared to the untreated control. Among all the treatments onion bulb extract (30.50q/ ha) showed the low yield. From the cost benefit ratio most benefit result was obtained in *Trichoderma viride* (1:2.26), Neem oil (1:1.96), Lemon grass oil (1:1.69) and Onion bulb (1:1.53). However, the present research findings are limited to one crop season under Prayagraj agro climatic conditions as such more trails are required in future to validate the findings.

References

1. Bhavani TV, Gohil VP, Patel JK. Isolation, Pathogenicity and Culture Media Study of *Helminthosporium maydis* Causing Maydis Leaf Blight Disease of Maize Advances in Life Sciences. 2016; 5(1):77-80.
2. Bugnicourt F. Deux especes nouvelles d' Helminthosporium isolates. DeRiz. Genn. Bot. 1955; 62:238-243.
3. Drechsler C. Leaf spot on maize caused by *Ophiobolus heterostrophus* N sp. The ascigerous stage of *Helminthosporium* exhibiting bipolar germination. Journal of Agriculture Research. 1925; 31:701-26.
4. Gerard Ezhilan J, Chandrasekhar V, Kurucheve V. Effect of six selected plant products and oil cakes on the selerotial production and germination of *Rhizoctonia solani*. Indian Phytopathlogy. 1994; 47(2):183-185.
5. Harish Kumar, Shafaat Ahmad, Sunil Zacharia. Efficacy of Fungal, Bacterial Bio-agents and Botanicals against Brown Spot (*Helminthosporium oryzae*) of Rice (*Oryzae sativa*) Research Journal of Chemical and Environmental Sciences. 2015; 3(2):27-31
6. Johnston A, Booth C. Plant Pathologists Pocket Book. Commonwealth Mycological Institute, Kew, Survey, England, 1983, 439p.
7. Malik VK, Singh M, Hooda KS, Yadav NK, Chauhan PK. Efficacy of Newer Molecules, Bioagents and

Botanicals against Maydis Leaf Blight and Banded Leaf and Sheath Blight of Maize. Plant Pathology Journal. 2018; 34(2):121-125.
8. Nisikado Y, Miyake C. Studies on two *Helminthosporium* diseases of maize caused by *H. turcicum* Pass and *Ophiobolus heterostrophus* Drechsler (*H. maydis* Nisikado and Miyake). Ohara Inst. Landwrit. Forsch. Ber. 1926; 3:221-26.
9. Payak MM, Sharma RC. Disease rating scales in maize in India. Technique of scoring for resistant to important diseases of maize. All India coordinated maize improvement project, Indian Agriculture Research Institute, New Delhi, 1982, 1-4p.
10. Purseglove JW, Osagie Eka. Tropical Crops: Monocotyledons. Longman Scientific and Technical, New York, 1992, 300-305p.
11. Sanjeev Kumar, Rani A, Jha MM. Potential of *Trichoderma spp* as bio-control agents against pathogens causing maydis leaf blight of maize. Journal of biological control. 2009; 23(1):89-91.
12. Santos GR, Cruz RB, Castro HG, Fidelis RR. Effect of essential oils of medicinal plants on leaf blotch in Tanzania grass. Scientific article. 2013; 44(3):587-593.
13. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1947; 150:850.
14. White DG. In: Compendium of Corn Diseases, 3rd ed., American Phytopathology. Soceity., St. Paul, MN, 1999.