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Siddu Lakshmi Prasanna
Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India

Priya John
Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India

Corresponding Author:
Siddu Lakshmi Prasanna
Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India

Exploring the proficiency of botanicals, bioagents and plant activators against maydis leaf blight of maize caused by *Bipolaris maydis*

Siddu Lakshmi Prasanna and Priya John

Abstract
Maize is one of the major food and forage crop grown globally and it is the most versatile crop adapted to different agro-ecological and climatic conditions. Maydis leaf blight is the destructive foliar disease in maize and to manage it biologically different natural available products were used for *in vitro* evaluation. Among the 11 botanicals tested, Ginger at 10 per cent concentration showed maximum inhibition of mean mycelial growth (72.68%) followed by Datura (69.59%), Sarpagandha (68.82%) and minimum inhibition in mycelial growth (30.21%) was recorded in Onion at 5 per cent concentration. Six biocontrol agents also evaluated for their antagonistic activity against *B. maydis* using dual culture technique and found maximum inhibition in mean mycelial growth in *T. harzianum* and *T. koningii* (78.97%) followed by *T. viride* (76.92%) and the least was found in *B. subtilis* (34.62%). Five plant defense activators were also evaluated for their antagonistic activity against *B. maydis* using by poisoned food technique at 100, 250 and 500 ppm. Among the different plant defense activators tested, at 500ppm concentration potassium phosphite was found most effective in inhibiting the mycelial growth of *B. maydis* with mean inhibition of 72.19 per cent followed by salicylic acid (70.04%), β-aminobutyric acid (64.42%) and least was found in benzoic acid (32.68%).

Keywords: Botanicals, poisoned food technique, antagonistic activity, dual culture technique, plant defense activators

1. Introduction
Maize is the most important cereal after rice and wheat. It is called as queen of the cereals or Miracle crop. It is one of the largest (49.33t) consumable cereals in the world. Its productivity is reduced due to the major stresses such as biotic and abiotic. Among biotic stresses, Maydis leaf blight incited by *Cochliobolus heterostrophus* anamorph *B. maydis* is a serious foliar disease and can cause up to 40–70% yield losses. Plant disease management using chemicals has adverse effect on environment and also there is possibility of resistance development by the pathogen. The disease is managed by application of fungicides which could lead to several environmental and health related problems (Atri *et al*., 2022) [2].
Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical strategies and limited chemical usage for an effective Integrated Pest Management (IPM) system (Monte, 2001) [9]. So ecofriendly management approaches are important mainly with the use of botanicals which are plentily available in the nature, plant defence activators which can induce systemic resistance and biocontrol agents which are efficient with high antagonistic activity. Keeping in view the present study was carried out to identify the effective botanicals, biocontrol agents and plant defence activators against maydis leaf blight of maize.

2. Material and Methods
2.1 *In vitro* evaluation of botanicals
Plant extracts used in the present study were given in Table 1. The plant extracts were prepared by cold water extraction method described by Shekhawat and Prasad (1971) [20]. *In vitro* evaluation of eleven plant extracts at two different concentrations *viz*., 5 and 10 per cent were studied against *B. maydis* using the poisoned food technique (Sharvelle, 1961) [12]. Per cent growth inhibition was calculated using the formula given by Vincent, 1947 [18].

Table 1: List of botanicals used for *in vitro* evaluation against *B. maydis*

Sr. No.	Common name	Botanical name	Family	Plant part used
1.	Neem	*Azadirachta indica* J.	Meliaceae	Leaves
2.	Tulsi	*Ocimum sanctum* L.	Lamiaceae	Leaves
3.	Garlic	*Allium sativum* L.	Amaryllidaceae	Cloves
4.	Ginger	*Zingiber officinalis R.*	Zingiberaceae	Rhizome
5.	Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome
6.	Jatropa	*Jatropha curcas* L.	Euphorbiaceae	Leaves
7.	Datura	*Datura stamoneum* L.	Solanaceae	Leaves
8.	Onion	*Allium cepa* L.	Amaryllidaceae	Bulb
9.	Adulsa	*Justicia adhatoda* L.	Acanthaceae	Leaves
10.	Ashwagandha	*Withania somnifera* L.	Solanaceae	Leaves
11.	Sarpagandha	*Rauwolfia serpentina* L.	Apocynaceae	Leaves and flowers
12.	Control	-	-	-

2.2 *In vitro* evaluation of biocontrol agents

In vitro evaluation of bioagents against *B. maydis* was carried out using dual culture technique (Martyn and Stack 1992). Per cent growth inhibition was calculated using the formula given by Vincent, 1947 [18].

2.3 *In vitro* evaluation of plant activators

In vitro evaluation of eleven plant activators at three different concentrations *viz*., 100, 250 and 500 ppm were studied agaisnt *B. maydis* using the poison food technique (Sharvelle, 1961) [12].

Per cent inhibition of growth of the test fungus was calculated by using the formula described by (Vincent, 1947) [18] A control was maintained in which the fungal pathogen was grown under similar conditions on agar medium.

$$\text{Per cent growth inhibition} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \times 100$$

Where,
C= Colony diameter in control
T = Colony diameter in treatment

3. Results and Discussion

The results on the efficacy of different botanicals and biocontrol agents were tested against maydis leaf blight are presented as under:

3.1 Efficacy of botanicals against maydis leaf blight

Eleven plant extracts were tested and observations on diameter of mycelial growth of *B. maydis* were recorded in the present study. The per cent inhibition of mycelial growth of the fungus by various botanicals was presented in Table 2, Figure 1 and Photo 1. Among eleven botanicals, Ginger (72.68%) was found to be effective and significantly superior in antagonistic activity at 10 per cent concentration. Next to Ginger, Datura (69.59%), Sarpagandha (68.82%), Turmeric (68.24%) which were more or less equally inhibiting the pathogen followed by Adulsa (62.74%) at 10 per cent concentration. Among the two concentrations assessed, 10 per cent was found effective and significantly superior over five per cent concentration. Onion was found to be least effective in both the concentrations.

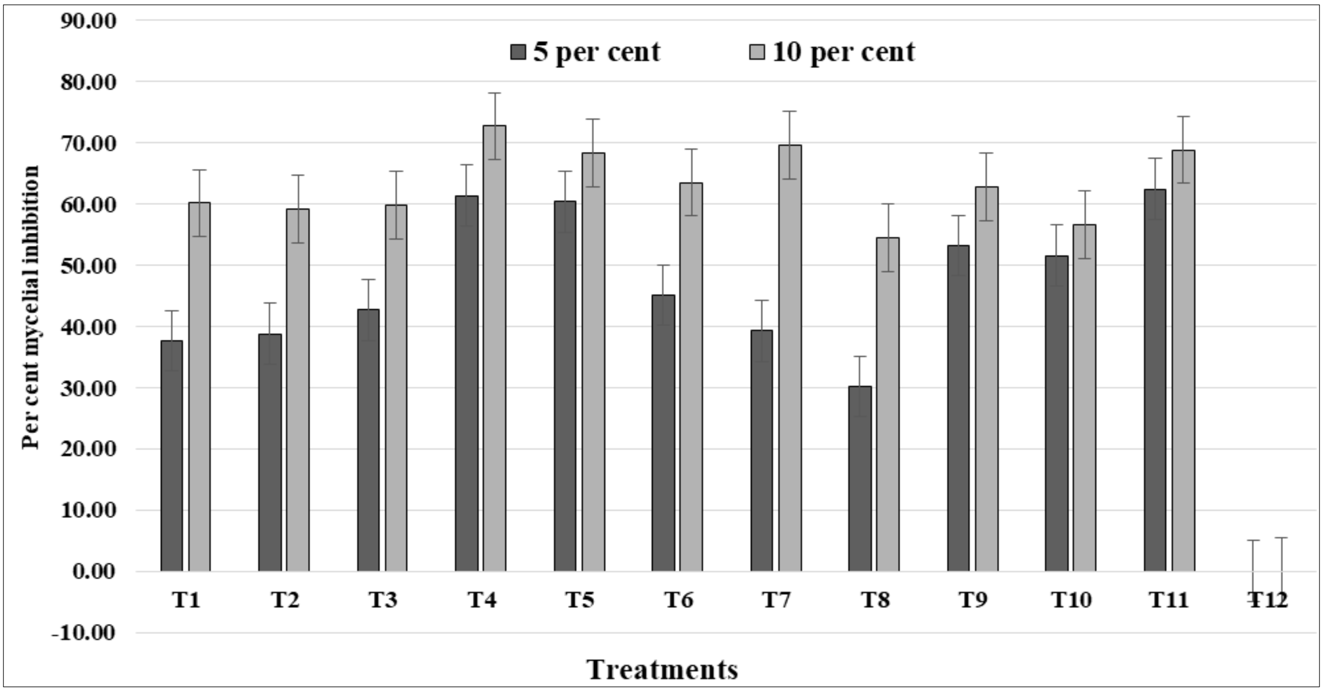
Botanicals possess the antimicrobial compounds such as Azadirachtin in neem (Verma *et al*., 2002) [16], sulfur compounds in garlic and onion, curcumin in turmeric, steroidal Saponins in ginger, Diterpenoids and alkaloids in datura (Mishra *et al*., 2020) [8] etc.

Jatoi *et al*., (2015) [5] recorded maximum inhibition of mycelial growth of *H. oryzae* in the ginger, garlic and datura extracts. Subedi *et al*., (2019) [15], Nayak and Hiremath (2019) [10], Kumar *et al*., (2009) [6] and Gurjar *et al*., (2012) [4] reported that neem (*A. indica*) and garlic were effective against *Helminthosporium* sp. in different crops. But in our study, we found ginger was effective at both concentrations followed by turmeric, datura and sarpagandha.

Table 2: Effect of botanicals against *B. maydis*

Sr. No.	Botanicals	Per cent growth inhibition		Mean
		5%	10%	
1.	Neem	37.64*(37.84) **	60.13(50.84)	48.88(44.34)
2.	Tulsi	38.80(38.52)	59.07(50.22)	48.93(44.37)
3.	Garlic	42.66(40.78)	59.84(50.67)	51.25(45.72)
4.	Ginger	61.38(51.58)	72.68(58.48)	67.03(55.03)
5.	Turmeric	60.32(50.96)	68.24(55.69)	64.28(53.33)
6.	Jatropa	45.07(42.17)	63.51(52.84)	54.29(47.50)
7.	Datura	39.28(38.81)	69.59(56.53)	54.44(47.67)
8.	Onion	30.21(33.34)	54.53(47.60)	42.37(40.47)
9.	Adulsa	53.18(46.82)	62.74(52.38)	57.96(49.60)
10.	Ashwagandha	51.54(45.88)	56.56(48.77)	54.05(47.32)
11.	Sarpagandha	62.45(52.21)	68.82(56.05)	65.63(54.13)
	S.Em.±	0.62	0.61	
	CD at 5%	1.82	1.77	
	CV %	2.21	2.89	

*Figures in outside parenthesis are original values,
**Figures in parenthesis are Arc sine transformed values



T1. Neem, T2. Tulsi, T3. Garlic, T4. Ginger, T5. Turmeric, T6. Jatropa, T7. Datura, T8. Onion, T9. Adulsa, T10. Aswagandha, T11. Sarpagandha, T12. Control

Fig 1: Effect of botanicals against *B. maydis*

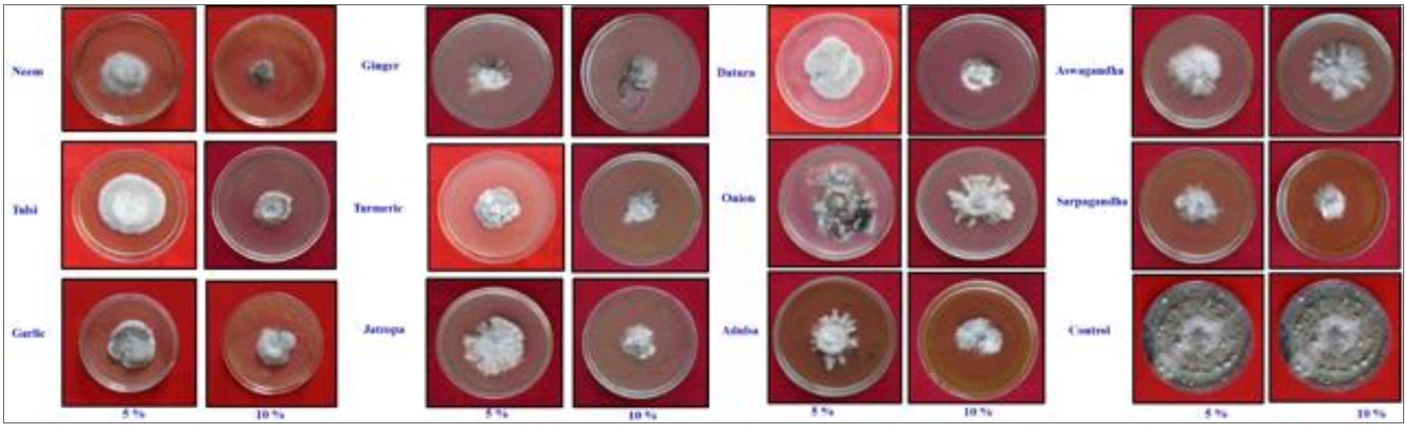


Photo 1: *In vitro* evaluation of botanicals against *B. maydis*

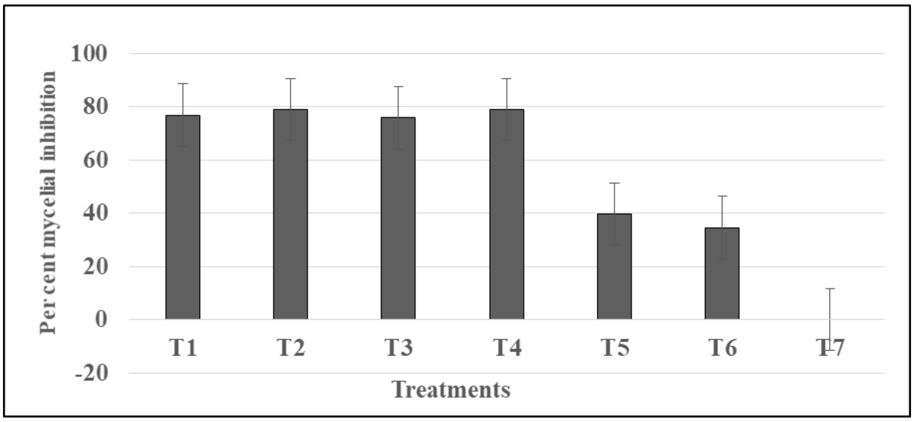
3.2 Efficacy of biocontrol agents against maydis leaf blight

In the present investigation, all the biocontrol agents reduced the mycelial growth of the test fungus *B. maydis* and presented in Table 3, Figure 2 and Photo 2. The per cent mycelial inhibition of *B. maydis* by biocontrol agents was found maximum in the treatment of *T. harzianum* and *T. koningii* (78.97%) followed by *T. viride* (76.92%) and the least was found in *B. subtilis* (34.62%). In treatments *T. harzianum* and *T. koningii* the mycelia were growing over the pathogen which indicates the faster growth and hyper parasitism of the biocontrol agent.

Table 3: Effect of biocontrol agents against *B. maydis*

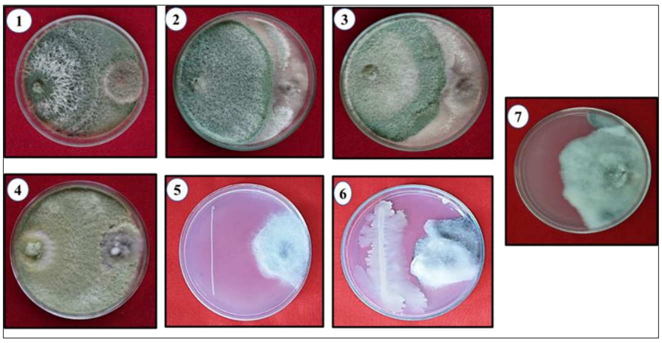
Sr. No.	Biocontrol agent	Colony diameter (mm)	Inhibition (%)
1.	*Trichoderma viride*	15.00	76.92*(61.28) **
2.	*Trichoderma harzianum*	13.67	78.97(62.70)
3.	*Trichoderma longibrachiatum*	15.67	75.89(60.59)
4.	*Trichoderma koningii*	13.67	78.97(62.70)
5.	*Pseudomonas fluorescens*	39.17	39.73(39.07)
6.	*Bacillus subtilis*	42.50	34.61(36.03)
7.	Control	65.00	-
	S.Em.±	0.45	
	CD at 5%	1.38	
	CV %	2.69	

*Figures in outside parenthesis are original values,
**Figures in parenthesis are Arc sine transformed values



T1. *T. viride,* T2. *T. harzianum,* T3. *T. longibrachiatum,* T4. *T. koningii,* T5. *Pseudomonas fluorescens,* T6. *Bacillus subtilis,* T7. Control

Fig 2: Effect of biocontrol agents against *B. maydis*



T1. *T. viride,* T2. *T. harzianum,* T3. *T. longibrachiatum,* T4. *T. koningii,* T5. *Pseudomonas fluorescens,* T6. *Bacillus subtilis,* T7. Control

Photo 2: *In vitro* evaluation of biocontrol agents against *B. maydis*

Trichoderma spp. has the ability to produce secondary metabolites such as antibiotics, acids and cell wall degrading enzymes (CDWE) which are potential enough to inhibit phytopathogenic fungi (Vinale *et al.*, 2008) [17]. Bacterial biocontrol agents have the ability to produce a wide range of secondary metabolic compounds of varied structure and function. The production of secondary antimicrobial compounds determines their capability to control many plant diseases (Silo-suh *et al.*, 1994) [13].

Similar results were also found in agreement where *T. harzianum* was found effective against turcicum leaf blight of maize caused by *Exserohilum turcicum* (Singh and Dutta, 2017; Viswanath *et al.*, 2017) [14, 19]. *T. viride* was found effective against *Bipolaris sacchari* in *in vitro* by Mane *et al.*, 2018 [6]. *T. viride* was found effective against turcicum leaf blight in *in vitro* by Subedi *et al.*, 2019 [15]. Kumar *et al.* (2018) also found different *Trichoderma* isolates against southern corn leaf blight of maize were promising and maximum inhibition in mycelial growth (93.48%) was recorded in RT-6 isolate followed by RT-9 isolate (91.25%), RT-7 (89.36%) and minimum inhibition in mycelial growth (73.94%) was measured in RT-1 isolate.

3.3 Efficacy of plant defense activators against maydis leaf blight

The relative efficacy of plant defence activators *viz.*, Salicylic acid, Benzoic acid, Potassium phosphite, β-aminobutyric acid and 2, 6-dichloroisonicotinic acid were evaluated at 100, 250 and 500 ppm concentration by Poisoned food technique. The observation regarding per cent growth inhibition were presented in Table 4, Figure 3 and Photo 3.

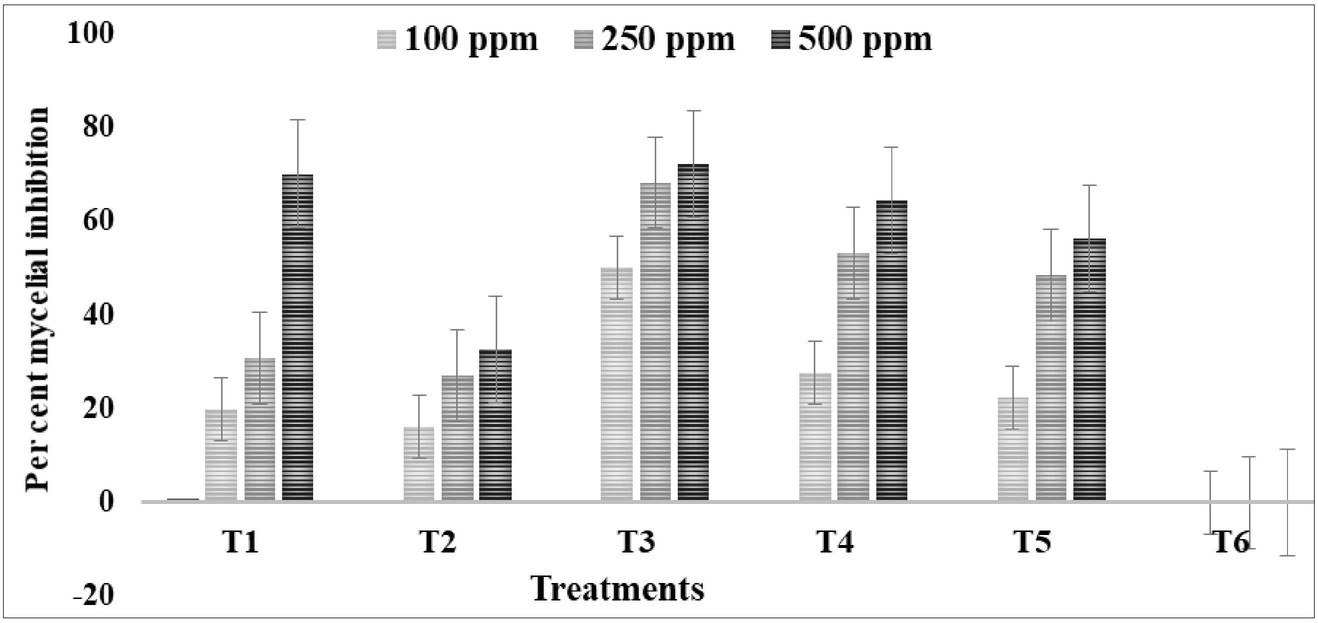
Table 4: Effect of plant defense activators against *B. maydis*

Sr. No.	Plant defense activators	Per cent growth inhibition			Mean
		100 ppm	250 ppm	500 ppm	
1.	Salicylic acid (SA)	19.85*(26.45) **	30.71(33.65)	70.03(56.81)	40.19(39.34)
2.	Benzoic acid	16.19(23.73)	27.15(31.4)	32.67(34.86)	25.34(30.22)
3.	Potassium phosphite (Kphi)	50.00(45.00)	68.25(55.7)	72.19(58.17)	63.48(52.82)
4.	β- aminobutyric acid (BABA)	27.71(31.76)	53.27(46.87)	64.41(53.38)	48.47(44.12)
5.	2,6-dichloro isonicotinic acid	22.47(28.29)	48.61(44.2)	56.24(48.58)	42.44(40.65)
	S.Em.±	0.64	0.60	0.58	
	CD at 5%	1.98	1.86	1.80	
	CV %	1.62	1.82	2.18	

*Figures in ouside parenthesis are original values,
**Figures in parenthesis are Arc sine transformed values

The results presented in Table 4 revealed that all the five plant defense activators at different concentrations (100, 250 and 500 ppm) found promising against *B. maydis*. Potassium phosphite at 500 ppm concentration showed high per cent growth inhibition (72.19%) followed by salicyclic acid (70.03%) and the least was found in benzoic acid (16.19%) at 100 ppm concentration. The five plant defense activators greatly varied in their efficacy to inhibit the growth of fungus under study.

From the results it is interfered that there was very less mycelial growth of the pathogen in potassium phosphote at all the different concentrations. The plant defense activator benzoic acid was found least effective at all concentration as compared to other fungicides.



T1. Salicylic acid, T2. Benzoic acid, T3. Potassium phosphite, T4. β-aminobutyric acid, T5. 2, 6-dichloroisonicotinic acid, T6. Control

Fig 3: Effect of plant defense activators against *B. maydis*

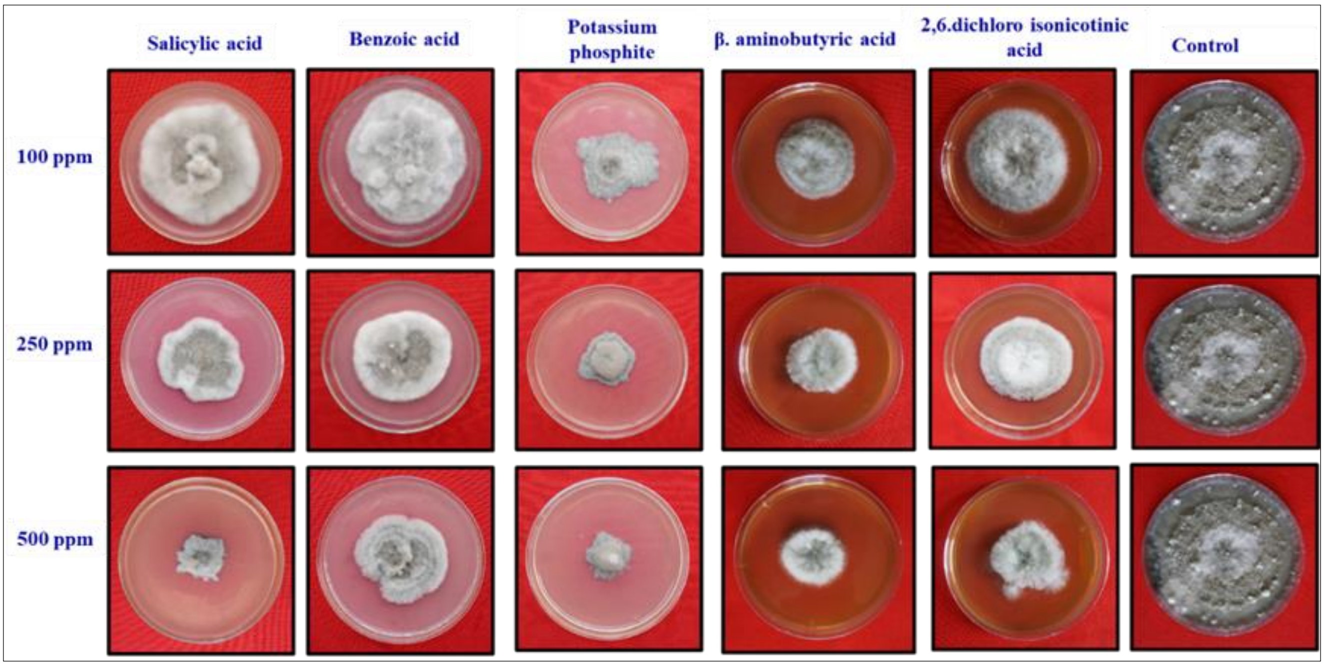


Photo 3: *In vitro* evaluation of plant defense activators against *B. maydis*

Plant defence activators does not act directly act against the fungus but it confers resistance in plants probably by inducing defence responses in the host tissue. Studies showed that BABA, SA, 2,6- Dichloro isonicotinic acid induces a variety of defence mechanisms including production of phenolics, peroxides, PR-proteins, callose, lignin and more, depending on the host-pathogen system (Reuveni *et al*., 2003; EL-Tanany *et al*., 2018; Archana *et al*., 2020) [11, 3, 1].

4. Conclusion

The studies of *in vitro* evaluation of botanicals, biocontrol agents and plant defense activators against *B. maydis* was carried out and the data revealed that Ginger at 10 per cent concentration showed maximum inhibition of mean mycelial growth (72.68%) followed by Datura (69.59%), Sarpagandha (68.82%) and minimum inhibition in mycelial growth (30.21) was recorded in Onion at 5 per cent concentration. In biocontrol agents maximum inhibition in mean mycelial growth was found in *T. harzianum* and *T. koningii* (78.97%) followed by *T. viride* (76.92%) and the least was found in *B. subtilis* (34.62%). Five plant defence activators were evaluated for their antagonistic activity against *B. maydis* using by poisoned food technique at 100, 250 and 500 ppm. Among the different plant defense activators tested, at 500ppm concentration potassium phosphite was found most effective in inhibiting the mycelial growth of *B. maydis* with mean inhibition of 72.19 per cent followed by salicylic acid (70.04%), β- aminobutyric acid (64.42%) and least was found in benzoic acid (32.68%).

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