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INTEGRATED MANAGEMENT OF *FUSARIUM* STALK ROT OF MAIZE CAUSED BY *FUSARIUM VERTICILLIOIDES*

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ABSTRACT

An experiment was laid to study the effect of four fungicides, four botanical extracts and three biocontrol agents *in vitro* against *F. verticillioides* and then under experimental field conditions to develop a suitable and effective management practice for this disease. Under *in vitro* conditions, Caviet (tebuconazole) completely inhibited the growth of the pathogen at all the concentrations. Similarly, neem oil at 0.2% (34.83%) and *Trichoderma harzianum* (52.92%) recorded highest inhibition among botanicals and bio-control agents. Under field experiment, treatment T_4, which is a combination of tebuconazole and *T. harzianum* recorded highest germination (91%), lowest mortality (7.33%), least disease incidence (19.33%), highest percent efficacy of disease control (80.66) and lowest average disease rating (1.6). Apart from reduced disease incidence, T_4 resulted into improved crop growth and yield. This combination of treatment can be used to reduce the incidence of post-flowering stalk rot of maize and improve the yield.

Key words : Tebuconazole, *F. verticillioides*, *T. harzianum*, Integrated management, botanicals

Introduction

Maize (*Zea mays* L.) is the third most important crop in India after rice and wheat. It is called as Queen of cereals due to its higher genetic yield potential. India ranks 4th in terms of total are and 7th in terms of production. The total production of maize in India during 2020-21 was 31.51 million tons with an area of 9.9 million hectares (ANGRAU Maize Outlook Report, 2021). Andhra Pradesh ranks first in India in terms of cropped area (3.01 lakh ha) and total production (17.84blakh tons) (www.indiastat.com). While in Uttarakhand, maize is grown over an area of 22 thousand hectares. The productivity in hills (976 kg/ ha) is less as compared to that of plains (1879 kg/ha). Dehradun district alone accounts for over one third of total maize area and production in the state (Khulbe *et al*., 2017). The production of maize is constrained by various factors including diseases. Among all the disease, post-flowering stalk rot of maize caused by *Fusarium verticillioides* is of major importance as it causes both qualitative and quantitative losses. It is also called as *Fusarium* stalk rot as *Fusarium* spp. are frequently associated with this disease. Loses caused by post-flowering stalk rot include discoloration of internal tissues, poor filling of ears, lodging of plants, stalk breakage and premature plant death (Khokhar *et al*., 2014). This disease affects the crop in between flowering and grain formation stages. When the stalks of affected plants are split opened, discoloration of pith can be seen (Dodd, 1980). This disease prevails in regions characterized by the presence of hot and dry weather conditions (Doohan *et al*., 2003). It was observed that use of any single management practice is not effective in controlling the stalk rot disease of maize and also too much use of chemicals leads to the development of resistance among fungi and harmful effect on non target organisms. Although, botanicals and bio-control agents have some antagonistic effect against plant pathogens, they are not very effective when used alone. So, there is an urgent need to augment an innovative approach for the control of post-flowering stalk rot disease by combining different components in eco-friendly and cost-effective manner (Integrated disease management) so as to reduce the disease incidence and improve crop yields.

Materials and Methods

In vitro testing of different chemicals, biocontrol agents and botanicals against *Fusarium verticillioides*

Pathogen responsible for the disease (*F. verticillioides*) was isolated from the infected stalks collected from the surveyed fields and pathogenicity was proved on maize hybrid Vipin 760. A total of four different fungicides *viz*., Bavistin (carbendazim 50% WP), Caviet (tebuconazole 25% WG), Indofil M-45 (Mancozeb 75% WP), Partner (Metalaxyl 8% + Mancozeb 75% WP) was tested against *F. verticillioides in-vitro* by poisoned food technique (Nene and Thapaliyal, 1993)at 5 different concentrations *viz*., 100, 200, 400, 800 and 1000 ppm. Four raw botanical extracts *i.e.*, garlic bulb extract, neem oil, marigold leaf extract and tulsi leaf extract were tested at 2 different concentrations (0.1% and 0.2%) using poisoned food technique. Similarly, three antagonists (*Trichoderma asperellum*, *T. harzianum* and *T. viride*) isolated from the surveyed fields were assayed against *F. verticillioides* by employing dual culture technique as suggested by Dennis and Webster (1971). All the *in vitro* experiments were carried out in completely randomized design with five replications. The colony diameter or radial growth of pathogen were measured at the end of 7 days after incubation and percent inhibition of mycelial growth was calculated according to the formula given by Bliss (1934). Compatibility between superior biocontrol agent, fungicide and botanical extract were carried out by employing poisoned food technique with slight modifications.

Integrated disease management of post-flowering stalk rot of maize

Based on the results from the *in-vitro* studies superior fungicide, botanical and biocontrol agent were selected and tested alone and in combinations under experimental field conditions on the incidence of post-flowering stalk rot maize at Uttaranchal University, Dehradun with a total of 7 treatments on Vipin 760 maize hybrid. The experiment was carried out in sick plots pre-inoculated with the pathogen and plants were inoculated with toothpick inoculation technique with slight modifications and intensity of the disease was recorded based on the disease rating scale given by Young (1943). Based on the observations, percent stalk rot, total lodging percent, percent disease index and percent efficacy of disease control were calculated.

Results and Discussion

Among all the fungicides tested, caviet recorded highest inhibition (100%) of radial growth of pathogen at all the tested concentrations. This was followed by bavistin which recorded 89.76% of growth inhibition of pathogen at 100 ppm and completely inhibited the growth of pathogen at other concentrations. Partner recorded least percent inhibition of pathogen at four different concentrations among the four fungicides and recorded a percent growth inhibition of 73% at 1000 ppm. Similar experiments were earlier performed by Khokhar *et al*. (2014) in which they tested the *in vitro* efficacy of 5 chemicals (bavistin, tebuconazole, dithane M-45, thiram and vitavax) against post-flowering stalk rot pathogen *Fusarium verticillioides* and reported that bavistin and tebuconazole completely inhibited the mycelial growth of pathogen at 400 ppm followed by thiram, mancozeb and vitavax. Similarly, Al-mamun *et al*. (2016) tested five fungicides and reported that bavistin completely inhibited the growth of pathogen *Fusarium oxysporum* at all the tested concentrations. Tebuconazole is a sterol biosynthesis inhibitor fungicide which have protective and curative properties (Odintsova *et al*., 2020).

All the four botanicals significantly inhibited the growth of the pathogen *in vitro*. The growth inhibition of pathogen by neem oil is 24.48% and 34.83% at 0.1% and 0.2% respectively, which was highest among the four. This was followed by garlic bulb extract which recorded percent growth inhibition of 17.04% at 0.2% concentration. Similar experiments were earlier performed by Sreenivasa *et al*. (2011) in which they reported that neem oil at a concentration of 2000 ppm (0.2%) recorded reduced radial growth (7.46 cm) of pathogen as opposed to that of control (8.95 cm). The antagonistic activity of neem oil may be due to the presence of bioactive compounds such as loeic acid, palmitic acid, stearic acid, linoleic acid, arachidic acid and propyl disulphide, which have antifungal and antibacterial properties (Khan *et al*., 2021; Swapna Sonale *et al*., 2018). Similar studies of using botanicals for the management of *Fusarium verticillioides* was reported earlier by Akinbode *et al*. (2014).

Among the three biocontrol agents, *Trichoderma harzianum* recorded maximum inhibition of radial growth of the pathogen (52.92%). This was followed by *T. viride* (45.75%) and *T. asperellum* (44.06%), which were statistically on par with each other. Similar results were reported by Veenstra *et al*. (2018) in which they isolated *Trichoderma asperellum* from maize seeds, which

Table 1 : *In vitro* evaluation of different fungicides, botanicals and antagonists against *Fusarium verticillioides*.

Fungicide/ Concentration (ppm)	Colony Diameter (cm)*				Percent growth inhibition*			
	Bavistin	Caviet	Indofil M-45	Partner	Bavistin	Caviet	Indofil M- 45	Partner
100	0.65	0	3.38	5.68	89.76	100	40.15	10.55
200	0	0	3.5	4.3	100	100	44.88	32.28
400	0	0	3.4	2.4	100	100	46.45	62.20
800	0	0	2.9	2.0	100	100	54.30	68.5
1000	0	0	2.5	1.7	100	100	60.62	73.00
Control	6.48	6.48	6.48	6.48	0	0	0	0
SEm±	0.04	0.13	0.004	0.18	0.06	2.31	0.05	2.96
CD at 5%	0.13	0.371	0.01	0.53	0.13	6.85	0.14	8.78

Mean of 5 replications.

Table 2 : *In vitro* evaluation of different botanicals against *Fusarium verticillioides*.

S. no.	Concentration (%)	Colony Diameter* (cm)				Percent inhibition*			
		Garlic bulb	Mari gold leaf	Neem oil	Tulsi leaf	Garlic bulb	Mari gold leaf	Neem oil	Tulsi leaf
1	0.1%	61.4	63	48.8	63.2	4.32	2.73	24.48	2.46
2	0.2%	58	57.4	42.2	58.7	17.04	11.34	34.83	9.41
3	Control	64.8	64.8	64.8	64.8	-	-	-	-
SEm±		0.1	0.1	0.1	0.12	0.69	1.61	2.30	2.56
CD at 5%		0.3	0.3	0.4	0.42	2.15	5.01	7.16	8.02

* Mean of five replications.

Table 3 : *In vitro* evaluation of different biocontrol agents on the radial growth of *Fusarium verticillioides*.

S. no.	Biocontrol agent	Radial growth (mm)*	Percent inhibition*
1	*Trichoderma asperellum*	31.5	44.06
2	*Trichoderma harzianum*	26.5	52.92
3	*Trichoderma viride*	30.5	45.75
4	Control	56.2	0
SEm±		0.12	1.69
CD at 5%		0.37	5.28

*Mean of five replications.

significantly inhibited the growth of *F. verticillioides* by 51.89%. Similar results were earlier reported by Sempere and Santamarina (2009), Błaszczyk *et al*. (2016) and Santos *et al*. (2017). The antagonistic activity of *Trichoderma* spp. *in vitro* is attributed to mycoparasitism which involves the production of antibiotic metabolites, cell wall degrading enzymes such as endochitinases, pectinases etc. (Harman *et al.*, 2004; Vinale *et al*., 2008; Mukerjee *et al*., 2022).

Compatibility tests

Compatibility tests results revealed that *Trichoderma harzianum* is compatible with the tested fungicide up to a concentration of 200 ppm and the minimum inhibitory concentration was found out to be 230 ppm. Similarly, *T. harzianum* was compatible with neem oil at 0.2% concentration.

Integrated management of post-flowering stalk rot of maize

Based on theresults from the*in vitro* studies, best fungicide (tebuconazole @ 0.4g/ 100g of seeds), botanical extract (neem oil @ 2ml/ 100g of seeds) and biocontrol agent (*Trichoderma harzianum* 10^8cfu/ gm) were selected and their individual as well as combined effects were evaluated under experimental field conditions (Table 4).

Germination (%)

Among different treatments, highest germination (91%) was recorded from the plants treated with combination of Tebuconazole and *Trichoderma harzianum* (T_4). This was followed by treatment T_1 (86.66%) in which plants were treated with tebuconazole alone. The lowest germination was recorded from the

Table 4 : Effect of different treatments on germination, percent mortality, disease incidence, percent efficacy of disease control and average disease rating.

Treatment No.	Treatments	Germination (%) *	Percent mortality at 70 DAS*	Percent Disease Index (PDI)*	Percent efficacy of disease control (PEDC)*	Average disease rating*
T_1	Crop + Tebuconazole	86.66	9.73	23.66	61.33	2.7
T_2	Crop + *Trichoderma harzianum*	81.66	16.00	36.66	42.10	3.2
T_3	Crop + Neem oil	72.00	19.00	40.66	32.00	3.7
T_4	Crop + Tebuconazole + *Trichoderma harzianum*	91.00	7.33	19.33	80.66	1.6
T_5	Crop + Neem oil + *Trichoderma harzianum*	72.66	15.30	28.00	34.67	3
T_6	Crop + Tebuconazole + Neem oil	82.00	12.21	22.66	66.72	2.6
T_7	Crop + *Fusarium verticillioides* (soil and stalk inoculated)	62.00	27.33	64.38	-	6.4
Control (uninoculated)		66.00	20.00	46.20	-	4
SEm±		1.65	0.88	3.32	1.11	0.20
CD at 5%		4.99	2.91	5.58	3.38	0.63

*Mean of 3 replications.

seeds treated with T_3 in which seeds were treated with neem oil alone whereas plants in the control plot recorded a germination of 66%.

Percent mortality at 70 DAS

Percent mortality was observed at 70 days after sowing and the results revealed that plants from treatment T_4 recorded lowest percent mortality rate (7.33%) followed by T_1 (9.73%) in which seeds were treated with tebuconazole alone. Highest mortality rate of 19% was recorded from the plot where plants were treated with neem oil alone (T_3). Plants from the inoculated and un-inoculated plots recorded a percent mortality of 27.33% and 20%, respectively.

Percent disease index (PDI)

Percent disease index were recorded and presented in Table 4. The results revealed that lowest percent disease index of 19.33% was recorded from treatment T_4. This was followed by T_6 and T_1, which recorded a percent disease index of 22.66% and 23.66%, respectively. Highest disease index of 40.66% was recorded from T_3 in which the seeds were treated with neem oil alone. Plants in the inoculated and un-inoculated plots recorded PDI of 64.38% and 46.2%, respectively.

Percent efficacy of disease control (PEDC) and average disease rating

Percent efficacy of disease control and average disease rating are always inversely proportional. Among different treatments, lowest average disease rating of 1.6 was recorded from treatment T_4 in which seeds were treated with tebuconazole and *Trichoderma harzianum* (Table 4). Similarly highest PEDC of 80.66% was recorded from T_4 (Table 4). This was followed by T6 and T_1, which recorded average disease ratings of 2.6 and 2.7 and PEDC of 66.72% and 61.33% respectively. The highest average disease rating of 3.7 and lowest percent efficacy of disease control of 32% was recorded from T_3. The plants from the inoculated and un-inoculated control recorded average disease rating of 6.4 and 4, respectively.

Effect of different treatments on growth and yield parameters of maize

The effect of different treatments on the growth and yield parameters of maize were recorded and presented in Table 5. Among all the treatments, highest plant height (206.33 cm), maximum number of internodes (11.8), highest cob length (19.53 cm), highest cob diameter (11.56 cm), maximum number of grains per cob (293) and highest test weight (207g) was recorded from T_4 in which

Table 5 : Effect of different treatments on the growth and yield parameters of maize.

Treatment No.	Treatment	Plant height (cm)*	No. of inter-nodes*	Cob length (cm)*	Cob diameter (cm)*	Grains/ cob*	Test weight (grams)*
T_1	Crop + Tebuconazole	165.33	10.56	16.63	10.33	285	206.66
T_2	Crop + *Trichoderma harzianum*	192.00	9.66	18.54	11.33	261	194.66
T_3	Crop + Neem oil	188.86	10.86	15.10	9.96	256	200.33
T_4	Crop + Tebuconazole + *Trichoderma harzianum*	206.33	11.80	19.53	11.56	293	207.66
T_5	Crop + Neem oil + *Trichoderma harzianum*	181.66	10.50	17.85	11.50	278	208.00
T_6	Crop + Tebuconazole + Neem oil	180.66	9.10	16.76	10.98	264	192.33
T_7	Crop + *Fusarium verticillioides* (soil and stalk inoculated)	159.00	8.56	13.93	9.60	249	184.00
Control (uninoculated)		176.33	10.56	17.50	10.86	257	192.00
SEm±		1.95	0.20	0.31	0.18	3.12	3.41
CD at 5%		5.9	0.60	0.95	0.56	9.44	7.54

*Mean of 3 replications.

the plants were treated with a combination of tebuconazole and *Trichoderma harzianum*. This was followed by treatment T_5 in which plants were treated with neem oil and *T. harzianum*. The lowest growth and yield parameters were recorded from T_3 in which the seed treatment was given with neem oil alone. Whereas the plants in the inoculated control plot recorded low growth parameters as a result of pathogen infection.

The results from the present investigation clearly state that different treatments have beneficial effects on maize plants when applied by combining them as compared to that of individual application. The treatment in which seeds treated with tebuconazole and *Trichoderma harzianum* clearly performed better with respect to reduced disease incidence and improved overall growth and yield parameters. There seems to be a synergistic effect when *T. harzianum* was applied in combination with tebuconazole. And also seeds treated with combination of neem oil and *T. harzianum* clearly recorded better growth and yield as compared to that of individual application. And also seeds treated with *T. harzianum* alone resulted into improved yield and growth as compared to that of untreated control. Neem oil although performed better among other botanicals *in vitro* and showed some effect under field conditions but couldn't cope up with other treatments. This may be due to lower persistence of azadirachtin.

Similar experiments were earlier conducted by Thori *et al.* (2012) in which they tested the combined effect of tebuconazole, bavistin, *Trichoderma viride* on the incidence of *Fusarium* stalk rotand reported that combination of bavistin and drenching of *T. viride* resulted into highest germination, higher percent efficacy of disease control and lower percent mortality at 70 DAS followed by combined application of tebuconazole and *T. viride*. Similar results were obtained when Khokhar *et al.* (2014) tested the combined application of *Trichoderma viride* and bavistin. Saravanakumar *et al.* (2017) recorded 86.66% of disease reduction of fusarium stalk rot when treated with *T. harzianum* under greenhouse conditions.

As a beneficial biocontrol fungi *Trichoderma* spp. can compete with the plant pathogens for space and nutrition on host plants thereby inhibiting the colonization of plant pathogens (Sood *et al.*, 2020). The antagonistic activity of the *Trichoderma* spp. may be attributed to secretion of diverse enzymes and secondary metabolites which can inhibit the growth of plant pathogens (VinodKumar *et al.*, 2017). *Trichoderma* spp. are also known to induce system resistance in treated plants against plant pathogens and other abiotic stresses (Gupta *et al.*, 2020). *Trichoderma* spp. is known to enhance growth by solubilization of phosphorous and other micro nutrients such as Fe, Mn, Mg etc. (Hoyos-Carvajal *et al.*, 2009). *Trichoderma* spp. are known to produce plant growth hormones such as auxins, gibberellins and cytokinin which further enhance the growth and development of treated plants (Rouphael *et al.*, 2020).

Conclusion

The outcome of the present experiments states that combination of fungicides with botanicals or biocontrol agents produced significantly better results as compared to that of their individual application. The combination of bioagents (*Trichoderma* spp.) and fungicides (tebuconazole) as seed treatment is economically viable and can be recommended for the management of post-flowering stalk rot of maize.

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