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Eco-Friendly Management of Alternaria Leaf Spot of Brinjal (*Solanum melongena* L.)

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A B S T R A C T

The present investigation was undertaken for the management of Alternaria leaf spot of brinjal using bio-agent, botanicals and a fungicide (treated check) were evaluated for their efficacy under *in vitro* and *in vivo* against Alternaria leaf spot of brinjal incited by *Alternaria solani.* The antimicrobial activity of four plant extractsNeem (*Azardirachta indica*), Onion (*Allium cepa*), Garlic (*Allium sativum*) and Datura (*Datura stramonium*) were tested against test fungus *Alternaria solani*. Garlic bulb extract showed minimum radial growth and maximum mycelial inhibition @ 5 and 15 per cent concentration. The dual culture result revealed that *Trichoderma harzianum* is an antagonistic to *Alternaria solani.* At 30, 60 and 90 DAT readings were taken for growth parameters and per cent disease intensity. Among the treatments, *Trichoderma harzianum* was found most effective in the percent disease intensity of brinjal, followed by Neem leaf extract as compared to carbendazim 50 WP (treated check) and control. The maximum yield were found to be in *Trichoderma harzianum*, followed by Neem leaf extract and Datura leaf extract as compared to Carbendazim (treated check) and control.

Introduction

Brinjal or Eggplant (*Solanum melongena* L.) is an important vegetable crop grown in varied parts of the world and is the second major vegetable crop next to potato in India. It is consider as the "King of Vegetables". It is a perennial plant which is herbaceous, erect or semi-spreading, and is usually grown as a seasonal crop in almost all the seasons (Shrivastava and Butani, 1998). It has many ayurvedic and medicinal properties, particularly "white" brinjal is said to be good for diabetic patients (Chaudhary, 1967).

Total production of Brinjal is about 32 million tonnes in the world wherein India is world's second largest producer after China

(Choudhary and Gaur, 2009). India's share is a cultivated area of 730.4 thousand hectares with an annual production of 12800.8 thousand MT and productivity of 17.5 MT per hectare wherein, Uttar Pradesh covered the cultivated area of 8.01 thousand hectares with an annual production of 275.40 thousand MT and productivity of 34.40 MT per hectare (National Horticulture Board, 2018).

The pathogen *Alternaria solani* is widely distributed and cause diseases in many crops. *Alternaria solani* infect all the above ground parts of the plant at all stages of growth and development (Peralta *et al.,* 2005). The mycelium consists of septa, branched, light brown hyphae which turned darker with age. The conidiophores are short, 50- 90µm long and dark in colour.

Conidia are beaked, muriform, dark colour and borne singly. Conidia contained 5-10 transverse septa and 1-5 longitudinal septa (Singh, 1987*).* Among different fungal diseases of brinjal, Alternaria leaf spot caused by *Alternaria solani* has become one of the most important disease of all brinjal varieties.

Loss due to the disease has been estimated up to 30-40 per cent. (Thippeswamy *et al.,* 2005). The disease in severe cases heavily damage the plants and cause heavy losses in fruit yield which lead to economic losses.

Bio-agent and botanicals belonging to various groups recommended for the management of Alternaria leaf spot of brinjal. Generally farmers are using only the chemicals for managing the disease, but it has negative impact on the environment and develop resistant in the pathogen.

Considering the effects of this disease, the present paper discusses the efficacy of *Trichoderma* sp. and botanicals for the management of Alternaria leaf spot of brinjal.

Materials and Methods

Preparation of Plant Extracts

Fresh leaves samples were washed in tap water and washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding equal quantity of water (1:1 w/v) just enough to moisten the samples so that it was easy to crush. The extracts were strained through the two layers of muslin cloth. Finally, filtrates thus obtained from the leaves were used as stock solution and was diluted further according to 5, 10 and 15% concentrations either in PDA or Sterile water in *in-vitro* and *in-vivo* respectively.

Poisoned Food Technique

The extract were poured in the PDA at 5%, 10 % and 15% respectively and the PDA was autoclaved. This extract media is then poured in the petri plate @20ml/petri plate. Five millimeter diameter disc of *Alternaria solani* was cut with the help of a sterilize cork borer and kept at the centre of each Petri plate containing the required concentration i.e. 5%, 10% and 15% of botanicals dissolved in PDA. Five replications were maintained. One plain PDA plate is kept along with it as control. The plates were incubated at ±28°Cand colony. Per cent inhibitions of mycelium growth were calculated by using the formula given by Vincent (1947).

$$\text{Per cent inhibition of colony (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Colony diameter in control

T = Colony diameter in treatment

Dual Culture Technique

A culture disc of 5mm of test fungus i.e. *Alternaria solani* was cut with the help of a sterilized cork borer and a same size of antagonistic was cut with the fresh sterilized cork borer and both was placed on the PDA plate opposite to each other at the periphery on the solidified PDA plate. As a control, the test fungus was placed on the centre of the PDA plate. The plates were incubated at ±28°C. The antagonistic percent inhibition was tested after the incubation period when the control plate is full of growth and the test fungus plate growth. Calculation was done by using the formula developed by Dickinson (1976).

PIRG = (R1-R2) / R1 x 100

R1 - Radius of *A. solani* colony in control plate;

R2 - Radius of *A. solani* colony in dual culture plate.

Field experiment

Observations recorded

Plant height (cm): Plant height was recorded at 30, 60 and 90 DAT.

No. of leaves: No. of leaves was recorded at 30, 60 and 90 DAT.

No. of branches: No. of branches was recorded at 30, 60 and 90 DAT.

Per cent disease intensity of Alternaria leaf spot during survey.

Observations on Alternaria leaf spot disease intensity were recorded on randomly selected plants from the bottom, middle and top leaves. The Alternaria leaf spot disease was graded on the basis of disease intensity observed on leaves by applying 0-9 disease rating scale developed by Mayee and Datar (1986).

Yield (tonnes/ha): Yield is calculated after the harvesting of the crop.

Benefit cost ratio.

Gross return was calculated by multiplying total yield with the market price of the produce. Cost of cultivation and cost of treatment imposition will be deducted from the gross returns, to find out net returns and cost benefit ratio by following formula (Reddy and Reddy, 2004).

$$B:C \text{ ratio} = \frac{\text{Net returns}}{\text{Cost of cultivation}}$$

Where,

B: C = Benefit Cost ratio

Results and Discussion

In vitro screening of *Trichoderma* spp. and plant extracts against *Alternaria solani*

The observations recorded *in-vitro* among the four plant extract, one fungicide and essential oil at 5, 10 and 15 % concentration. The radial growth of test fungus is high (34.33mm) at 5% conc. of datura while the growth of fungus is moderate (33mm) at 10%conc. of datura. Garlic showed lesser growth of test fungus in comparison to neem leaf extract. Garlic bulb extract show maximum inhibition at 15%conc. inhibiting 78.89% of test fungus, followed by Eucalyptus oil and Carbendazim (50 WP) at 15%inhibiting 77.78 and 73.89% respectively. Similar effect of the botanicals against *A. solani* were reported earlier by several workers (Patni and Kolte, 2006; Singh and Singh, 2007).

Table.1 Effects of plant extract and essential oil on radial growth and per cent inhibition of *A. solani*

Treatment No.	Treatment Details (T)	Mean colony diameter*				Per cent Inhibition			
		Concentration (C)				Concentration (C)			
		5%	10%	15%	Mean (T)	5%	10%	15%	Mean (T)
T_2	Neem Leaf Extract	29.50	27.50	25.00	**27.33**	67.22	69.44	72.22	**69.63**
T_3	Garlic Bulb Extract	24.50	22.00	19.00	**21.83**	72.78	75.56	78.89	**75.74**
T_4	Datura Leaf Extract	34.33	33.00	30.00	**32.44**	61.86	63.33	66.67	**63.95**
T_5	Onion Bulb Extract	32.33	30.33	29.50	**30.72**	64.08	66.30	67.22	**65.87**
T_6	Eucalyptus oil (0.01%)	22.00	21.00	20.00	**21.00**	75.56	76.67	77.78	**76.67**
T_7	Carbendazim (50 WP)	26.33	25.50	23.50	**25.11**	70.74	71.67	73.89	**72.10**
T_0	Control	90.00	90.00	90.00	**90.00**	0.00	0.00	0.00	**0.00**
	Mean (C)	**37.00**	**35.62**	**33.86**		**58.89**	**60.42**	**62.38**	
								S.Em. (±)	**C.D. (5%)**
	Treatment							0.80	2.33
	Concentration							0.43	1.24
	Int. (T X C)							1.13	3.29

***** Average of five replicates

Table.2 Effect of bio-agent against mycelial inhibition of *A. solani*

% inhibition in colony diameter				
Sr. No.	**Treatment details**	**Colony diameter (mm)**	**Inhibition %**	**Mean**
1	*Trichoderma harzianum*	20	77.66	48.83
2	*Trichoderma viride*	22	75.50	48.75
3	Control	90	0	45
S.E(m)				0.48
C.D. (5%)				2.31

***** Average of five replicates

Table.3 Effects of treatment on plant height, number of leaves and number of branches

Treatments		Dosage	30 DAT			60 DAT			90 DAT		
			Plant height	Leaves	Branches	Plant height	Leaves	Branches	Plant height	Leaves	Branches
T_0	Control	-	16.66	9.33	2.60	33.26	26.66	8.20	37.86	44.80	**12.13**
T_1	*Trichoderma harzianum*	10%	24.30	11.87	4.07	35.86	31.60	9.67	40.97	49.07	**14.47**
T_2	Neem leaf extract	5%	21.13	11.33	4.00	35.50	29.00	9.28	38.83	47.27	**13.33**
T_3	Garlic bulb extract	10%	19.26	10.60	3.27	35.33	30.86	8.87	39.26	46.93	**13.47**
T_4	Datura leaf extract	5%	21.46	11.80	3.69	35.40	30.33	8.93	38.40	47.53	**13.40**
T_5	Onion bulb extract	5%	20.66	10.60	3.53	35.43	28.66	9.27	38.50	47.93	**13.87**
T_6	Eucalyptus oil(0.01%)	2%	16.86	10.00	3.13	34.33	29.17	8.67	38.66	45.47	**12.33**
T_7	Carbendazim (50 WP)	0.2%	23.23	10.75	3.67	34.73	31.00	9.07	40.60	47.87	**14.40**
C. D. (5%)		-	**1.69**	**1.09**	**0.39**	**1.41**	**2.84**	**0.51**	**1.06**	**1.64**	**0.44**
S. Ed (±)		-	**0.78**	**0.50**	**0.18**	**0.65**	**1.31**	**0.24**	**0.49**	**0.76**	**0.24**

***** Average of three replicates

Table.4 Effect of treatments on disease intensity (%) in brinjal

Tr. No.	Treatment	Disease intensity (%)		
		30DAT	60DAT	90DAT
T_0	Control	13.33	22.33	33.33
T_1	*Trichoderma harzianum*	6.67	18.30	26.67
T_2	Neem leaf extract	7.33	18.33	27.33
T_3	Garlic bulb extract	8.33	20.67	28.33
T_4	Datura leaf extract	9.67	19.67	29.67
T_5	Onion bulb extract	9.00	20.00	30.67
T_6	Eucalyptus oil(0.01%)	10.67	18.67	29.33
T_7	Carbendazim (50 WP)	6.33	16.00	24.67
	C.D. (5%)	**1.85**	**1.88**	**2.37**
	S.Ed (±)	**0.85**	**0.86**	**1.09**

***** Average of three replicates

Table.5 Effects of treatment on yield

Treatments	Yield (t/ha)	Total cost of yield	Total cost of cultivation	C:B ratio
Control	14.50	217500.00	60395	**1:2.60**
Trichoderma harzianum	16.67	250050.00	60605	**1:3.13**
Neem leaf extract	16.25	243750.00	61445	**1:2.97**
Garlic bulb extract	16.08	241200.00	61895	**1:2.90**
Datura leaf extract	16.58	248700.00	61395	**1:3.05**
Onion bulb extract	15.83	237450.00	61045	**1:2.89**
Eucalyptus oil(0.01%)	15.67	235050.00	61145	**1:2.84**
Carbendazim (50 WP)	**16.75**	**251250.00**	**60655**	**1:3.14**

Fig.1 Effect and interaction of plant extracts and essential oil on radial growth of *A. solani*

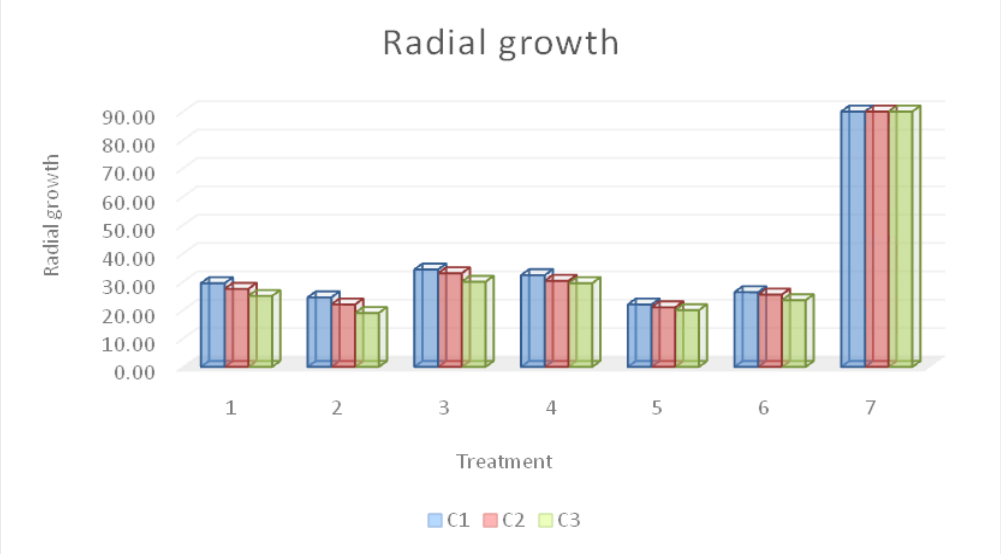


Fig.2 Effect of bio-agents on percent mycelial inhibition of *Alternaria solani*

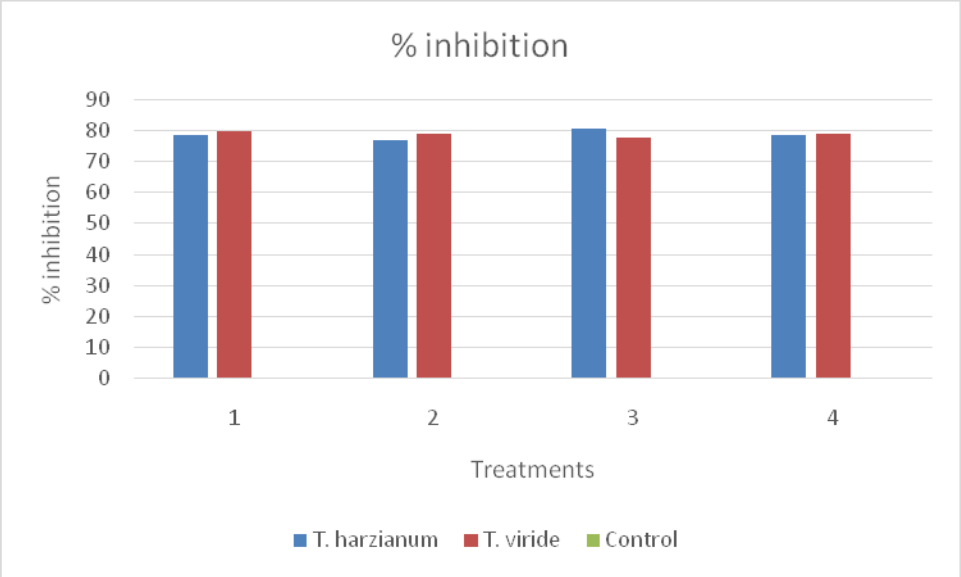


Fig.3 Effects of treatment on plant height, number of leaves and number of branches

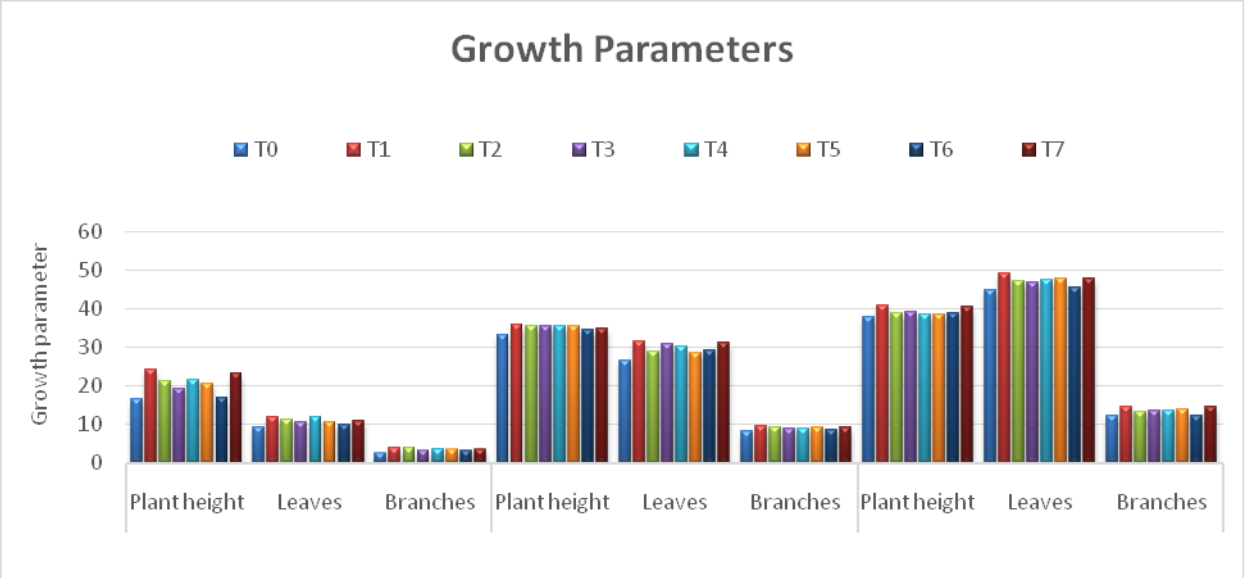


Fig.4 Effect of treatments on disease intensity (%)

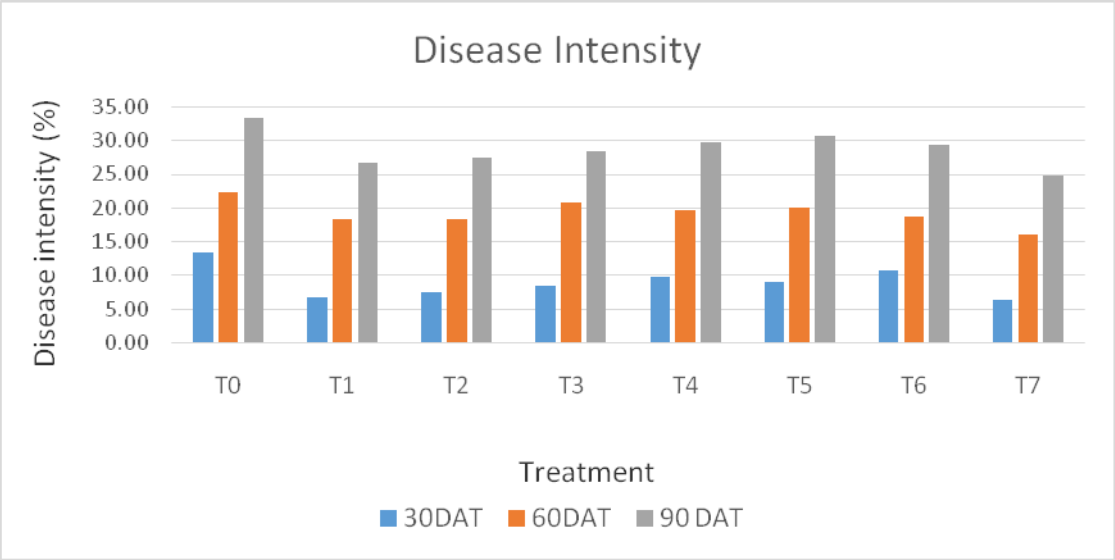
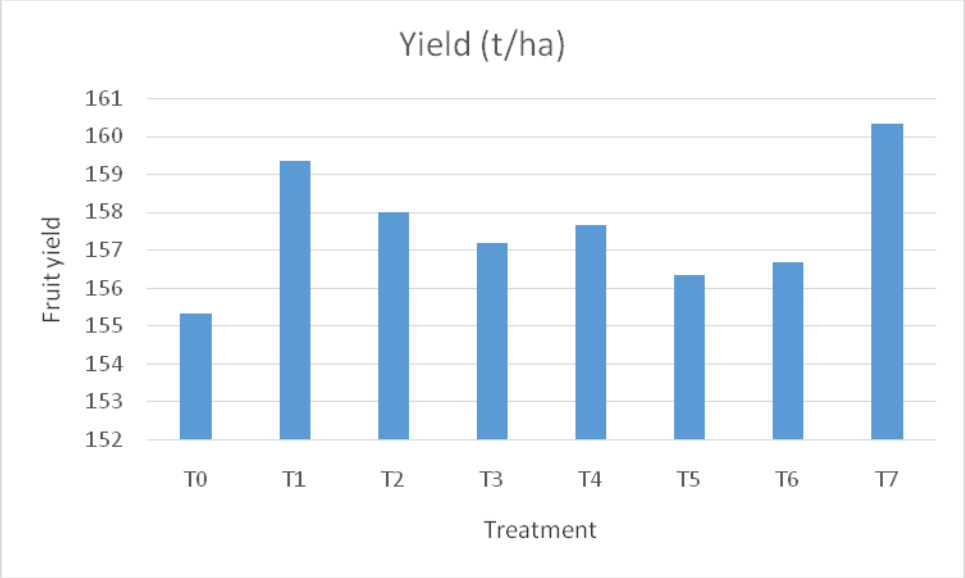


Fig.5 Effect of treatments on yield



Maximum inhibition of mycelial growth of fungus was recorded 77.66% and minimum growth was 75.50%.Similar result were recorded 70%, 78% by Sempere and Santamarina (2007).

Field evaluation

Effect of treatments on plant growth parameters

The observations were recorded at 30, 60 and 90 DAT for growth parameters and percent disease intensity. Growth parameters included plant height, branches and leaves.

Treatment T_1*T. harzianum* was found the best among all the treatments giving best height and more number of branches.

Maximum plant height(cm) was recorded with T_1(40.97 cm) followed by T_8 (40.60 cm) and T_4 (39.26 cm). Maximum number of

leaves(49.07) was recorded with T_1, followed by $T_6$47.93 and $T_8$47.87. Maximum number of branches(14.47) was recorded with T_1, followed by $T_8$14.40 and $T_4$13.87. In the present studies, number of branches at 30, 60 and 90 DAT was recorded in *Trichoderma harzianum* followed by Neem leaf extract was found to be effective over other treatments. This similar result was found in chilli by Gollagi (1999) and in tomato by Mehta *et al.*, (1989).

Effect of treatments on disease intensity

The minimum per cent disease incidence (%) at 30 DAT was recorded with *Trichoderma harzianum* (6.67%) and Neem leaf extract (7.33%). The minimum per cent disease intensity (%) at 60 DAT was recorded with *Trichoderma harzianum* (18.30%) and Neem leaf extract (18.33%).

The minimum per cent disease intensity (%) at 90 DAT was recorded with *Trichoderma harzianum* (26.67%) and Neem leaf extract (27.33%). This results were similar to the findings of Raja *et al.*, (2007).

In the studies conducted by Raja *et al.*, (2007), minimum disease intensity was found with *Trichoderma harzianum* through seed treatment was 17.78% at 60 DAT and effective over other treatments.

Effect of treatments on yield and C:B ratio

Maximum grain yield of brinjal (t/ha) was recorded in T1 *Trichoderma harzianum* i.e. 16.67 and i.e. T4 Neem leaf extract 16.25 as comparison of control (14.50). The economics analysis of the data over the session that T_1 *Trichoderma harzianum* gross returns Rs. 250050.00, net returns Rs. 189445.00 with C:B ratio 1:3.13 as compared to control gross returns Rs. 217500.00, net returns Rs. 157105.00 with C:B 1:2.60.

The present study, it was found that Neem leaf extract was most effective against *Alternaria solani,* causing Alternaria leaf spot of brinjal. Neem leaf extract followed by Datura leaf extract and *Trichoderma harzianum* were significantly superior in the treatment. The results of the present experiment are limited to one crop season under Prayagraj agro-climatic conditions, as such to validate the present findings more such trials should be taken up in future.

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