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Management of spot blotch of wheat using inducer chemicals under field conditions

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ABSTRACT

An experiment was conducted for two consecutive years (2012-13 and 2013-14) to determine the effect of different inducer chemicals on spot blotch disease of wheat and its impact on grain yield. All the inducer chemicals reduced the spot blotch infection significantly at different concentration levelsand also increase the yield attributing characters like number of grains/panicle and 1000 seed weight. The accumulation of phenol increased up to 96 days after sowing (DAS), whereas, peroxidase accumulation increased up to 68 days after sowing in wheat plants in all the treatments.Among the different inducer chemicals salicyclic acid (10^{-4} M) and CuSO4 (10^{-4} M and 10^{-5} M) showed good results even at low concentration levels. Therefore, the inducer chemicals can be used as an alternative method to manage the spot blotch of wheat.

Keywords: Spot blotch, inducer chemicals, defense, SAR and wheat

1. Introduction

India witnessed record wheat production of 98.61 million tons during 2017–2018 with all time highest productivity of 3318 kg/ha. Contrarily, the percentage decline in acreage was highest in case of West Bengal (-64.24%), followed by Maharashtra and Telangana (ICAR-IIWBR, 2018). The wheat scenario in North Eastern Plain Zone of India is more sensitive due to higher intensity of spot blotch disease which further causes more yield losses in the late sown wheat crops. So, our research effort should be focused on North eastern plain zone to increase the yield by minimizing disease severity. Yield loss was predicted to be 18-22% in India (Singh and Srivastava, 1997). Recently, it has already been measured that there was a 1.8–2.0 kg ha^{-1} decrease in grain yield and 0.89–1.59 g decrease in 1000 grain weight with every one percent increase in disease severity of spot blotch (Devi *et al.*, 2018) and it could be more at farmers fields in the eastern Gangetic plains. Grain yield loss due to spot blotch in South Asia ranged from 4-38% and 25-43% respectively in the year 2004 and 2005 (Duvellier *et al.*, 2005).

In past few decades, researchers were mostly focusing on use of chemical fungicides with different modes of action to decrease the yield loss caused by the spot blotch (Pasquer *et al.*, 2005; Mahapatra and Das, 2013; Singh *et al.*, 2014). Management of spot blotch of wheat is difficult as the fungus is seed-borne (causes black tip), soil borne as well as air borne. However, many alternative strategies have been find out to combat this disease like use of resistant varieties, fungicidal spray, choice of sowing date, sowing healthy seeds, etc. (Hetzler *et al.*, 1991; Villareal *et al.*, 1995; Mahto, 1999; Hossain and Hossain, 200; Devi *et al.*, 2012, Kumar *et al.,* 2019).

Inducer chemicals represents a natural and ecological approach for controlling diseases and provides many distinctive benefits to farmers and eco-friendly for environment, as they degrade quickly, when it applies as seed treatment and reduce the risk of residual effects on food (Gao *et al.*, 2014). According to Xue *et al.,* 1998, peroxidases and total phenol content are the main enzymes which involved in phenyl-propanoid metabolism. Several inducer chemicals other than fungicides such as *Salicylic acid*, Benzothiadiazole and Isonicotinic acid etc. have been found effective inducing systemic acquired resistance (SAR) compound in plants play an important role against pathogens (Agrios,1997; Vallad and Goodman, 2004). In wheat and barley crop Benzothiadiazole already has been tested as inducing resistance against powdery mildew

(Pasquer *et al.*, 2005; Jonczyk and Smagacz, 1999). However, Copper sulphate, Zinc sulphate, Methionine, Isonicotinic acid, 2,4-D, Sodium Selenite have never been tested against this disease.

The main objective of this study was to evaluate *in vivo* effects of some new inducer chemicals formulations against the *B. sorokiniana*in wheat, compare the effects of different formulations of inducer chemicalson disease resistance and determine their effects on grain yield.

2. Materials and methods:

2.1. Experimental field:

The field trials were conducted for two consecutive years during winter 2012-13 and 2013-14 at District Seed Farm,Bidhan Chandra Krishi Viswavidyalaya (BCKV),Kalyani, Nadia, West Bengal, India.The susceptible wheat cv. Sonalikarecommended for NEPZ of India, was used for experimental purposes. Experiment was designed in randomized block design with plot size 3x2m^2. There were 10 rows of 2m length with inter-row spacing 25cm ineach plot.

2.2. Treatments

All these compounds are analytical grade and used for seed treatment before sowing in the field.

2.3. Foliar Inoculation

Foliar inoculation was done by spraying the conidial suspension (3 x 10^4 spore/ml) of *B. sorokini ana* on the wheat plants. The inoculation was done in morning hours (8:00-9:00 a.m.). Before inoculation, the plots were thoroughly irrigated for creating a moist environment. The conidial suspension was sprayed on leaves uniformly with an atomizer. Foliar inoculation was done at two stages of plant growth*i.e.* tillering and boot leaf stages.

SL.No	Treatment	**Concentration**
T1	Copper sulphate	10^{-4} M
T2	Copper sulphate	10^{-5} M
T3	Zinc sulphate	10^{-4} M
T4	Zinc sulphate	10^{-5} M
T5	DL-Methionine	10^{-2} M
T6	DL-Phenylalanine	10^{-3} M
T7	L-Cysteine	10^{-3} M
T8	Salicylic acid	10^{-4} M
T9	Isonicotinic acid	10^{-4} M
T10	2,4-D	10^{-6} M
T11	Reducing agent (Sodium selenite)	10^{-4} M
T12	Untreated control	

2.4. Sample collection and assessment of disease severity

Post-inoculation, Leaf samples were collected randomly from selected plants representingall three replications of a treatment. Samples were collected at the interval of 14 days (40,54,68,82 and 96 days after sowing, DAS) to determine the changes in defense-related enzymes, protein concentration and total soluble phenolic content. Timing of samplecollection was between 10:00 a.m. to 12:00 p.m.

2.5. Double Digit rating scale for spot blotch of wheat

The proposed rating system has digits rating 0-9 and severity is recorded on the top two leaves of the plant at 59-65 growth stages at Zadok's scale. The spot blotch severity was taken in percent leaf area covered separately for flag (F) and leaf below (F-1).

2.6. Disease severity:

The calculation of disease severity was performed as percent disease leaf area (DLA%). The 10 plants per replications were randomly selected and DLA% per replication was calculated using the following formula,

Percent diseased leaf area (DLA%) = a/9× b/9 X100

2.7. Estimation of total Phenol

The total phenol content of wheat leaf was determined using Folin-Ciocalteau Reagent (FCR) (Vinson *et al.,* 1998).To determine the conjugated and un-conjugated ('total') phenol present in wheat leaf, 0.5g sample was taken and total phenol was extracted with 15 ml of 1.2 N HCl in 50% aqueous methanol. It was heated at 72-80° C for 1 hour. After cooling, extracted material was centrifuged at 10,000 rpm for 30 minutes. Supernatant was decanted off in a graduated tube and was diluted to 25 ml with 1.2 N HCl in 50% aqueous methanol. For the estimation of total phenol, 0.2 ml of aliquot was diluted with distilled water to make a final volume of 3 ml in a test tube, 0.5 ml FCR was added to it. After 3 minutes, 2 ml of 10% sodium carbonate was added. Shaked the test tube well and then warmed it at about 50-60° C for 8-10 minutes in a water bath. The solution was cooled and the absorbance was read at 650 nm.Total phenol content was determined using a standard curve made from gallic acid. The total phenol content is expressed as mg gallic acid equivalent per gram dry matter (mg GAE/g DM).

Table 1: Mean Disease leaf area percentage (DLA%) under induced resistance on wheat grown in two consecutive years (2012-14)

Treatments		Percent leaf infection (DLA %)		
		2012-13	2013-14	Pooled
T_1	$CUSO_4$ (10^{-4}M)	15.23 (23.30)	14.40 (22.62)	14.81 (23.04)
T_2	$CUSO_4$ (10^{-5}M)	17.28 (24.92)	16.46 (24.24)	16.87 (24.63)
T_3	$ZnSO_4$ (10^{-4}M)	42.22 (40.81)	40.33 (39.69)	41.28 (40.27)
T_4	$ZnSO_4$ (10^{-5}M)	40.33 (39.69)	37.45 (38.00)	38.89 (38.87)
T_5	DL-Methionine (10^{-2}M)	24.86 (30.21)	23.05 (28.97)	23.95 (29.64)
T_6	DL-Phenylalanine (10^{-3}M)	22.63 (28.70)	20.58 (27.28)	21.60 (28.04)
T_7	L-Cysteine (10^{-3}M)	29.96 (33.48)	27.98 (32.19)	28.97 (32.88)
T_8	Salicylic acid (10^{-4}M)	13.17 (21.68)	12.35 (21.00)	12.76 (21.35)
T_9	Isonicotinic acid (10^{-4}M)	37.45 (38.00)	35.14 (36.58)	36.30 (37.34)
T_{10}	2,4 D (10^{-6}M)	40.33 (39.69)	37.04 (37.63)	38.68 (38.75)
T_{11}	Sodium Selenite(10^{-4}M)	29.22 (32.83)	27.98 (32.19)	28.60 (32.65)
T_{12}	Untreated control	59.26 (50.67)	56.79 (49.20)	58.02 (49.91)
	SEM (±)	1.75	1.91	1.30
	CD at 5%	5.14	5.62	3.70

2.8. Peroxidase (POD) activity

Peroxidase estimated as per the method of Shannon *et al.,* (1966). The enzyme was extracted by grinding 1.0g fresh leaf tissue with 10ml of 0.1 M Sodium Phosphate buffer, pH 7.5 containing 2% PVP (polyvinylpyrrolidone) and 0.25 % Triton-X, in a pre-chilled mortar and pestle. The extracted sample was centrifuged at 10,000 rpm for 30 minutes at 4°C and the supernatant was used as the enzyme source which was stored in an ice bath until the assay was carried out. Peroxides was estimated by mixing 0.05 ml chilled enzyme extract with 2.8 ml reaction mixture (4% Guaiacol dissolved in methanol – 0.15 ml, 2.65 ml sodium potassium buffer (0.1M) pH 7.5). Reaction was initiated by adding 0.15ml of H_2O_2 (1%). The change in activity was measured at 470nm. Initial absorbance was read and then at every 30 seconds interval upto 3 minutes. Enzyme activities were expressed as micro mole of Guaiacol oxidized/min/g of leaf sample.

3. Results and discussion

3.1. Diseases leaf area percentage (DLA %)

Chemical inducers at their different concentration levels significantly reduced the percent of leaf infection in all treatments in comparison to untreated control in both years (Table 1). The two years pooled mean data also showed the similar type of disease reaction pattern.Minimum DLA% was noticed in plants treated with salicylic acid 10^{-4} M (12.76 %), statistically at par with $CuSO_4$ 10^{-4} M (14.81 %) but differs with $CuSO_4$ 10^{-5} M (16.87 %). Contrarily, maximum DLA% was noticed in plants treated with $ZnSO_4$ 10^{-4} M (41.28 %),statistically at par with $ZnSO_4$ 10^{-5} M (38.89%) and 2, 4-D 10^{-6}M (38.68 %). Isonicotinic acid 10^{-4} M (36.30 %) showed no significant difference in severity of DLA% from chemicals like 2, 4-D 10^{-6} M and $ZnSO_4$ 10^{-5}M. Medium DLA% was noticed with DL-phenylalanine 10^{-3} M (21.60 %), statistically, at par with DL-methionine 10^{-2} M (23.95 %) but differ with sodium selenite 10^{-4} M (28.60 %) and L-cysteine 10^{-3} M (28.97 %). All the chemicals at their different concentration levels, significantly reduced the percent of leaf infection in comparison to untreated control. The inducer chemicals can reduce the disease severity

Table 2: Variation in total phenol content under induced resistance against leaf blight of wheat at different days after sowing (DAS)

Treatments	Total phenol (mg/g of sample) at different days after sowing					
	40 DAS	54 DAS	68 DAS	82 DAS	96 DAS	Mean
$CuSO_4$ (10^{-4}M)	4.13	4.21	4.26	4.43	5.66	4.54
$CuSO_4$ (10^{-5}M)	3.20	3.26	3.59	4.39	5.50	3.99
$ZnSO_4$ (10^{-4}M)	2.63	2.77	2.84	3.59	4.38	3.24
$ZnSO_4$ (10^{-5}M)	2.60	2.76	3.20	3.89	4.62	3.41
DL-Methionine (10^{-2}M)	2.94	2.99	3.51	4.27	5.15	3.77
DL-Phenylalanine (10^{-3}M)	3.03	2.98	3.55	4.30	5.41	3.86
L-Cysteine (10^{-3}M)	2.79	3.25	3.21	3.91	4.74	3.58
Salicylic acid (10^{-4}M)	4.15	4.49	4.16	4.45	5.67	4.59
Isonicotinic acid (10^{-4}M)	2.66	2.93	3.26	4.09	5.00	3.59
2,4 D (10^{-6} M)	2.67	2.93	3.22	4.16	4.64	3.52
Sodium Selenite (10^{-4}M)	2.73	2.83	3.34	4.26	4.81	3.60
Untreated control	2.43	2.52	2.40	3.32	4.30	2.99
MEAN	3.00	3.16	3.36	4.09	4.99	
	SEM (±)			CD at 5%		
Date	0.025			0.070		
Treatment	0.061			0.171		
Date x Treatment	0.086			0.241		

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by increasing resistance in plants. Dasgupta *et al.* (1999) reported that seed treatment with $ZnSO_4$ 10^{-3} M significantly control the collar rot of groundnut caused by *Aspergillus niger*. These results were in concordance of Mosa (2002), Geetha and Shetty(2002) and Behera *et al.*,(2017). Oostendorp*et al.,* (2001) reported that the salicylic acid can induced systemic acquired resistance (SAR) in plants.

Biochemical changes associated with defense mechanism

3.2. Total phenol

Total phenol content was increased significantly in all the treatments at their different concentration levels in comparison to untreated control. It was also observed that with the increase in the age of the plant, there was a significant increase in total phenol content. The total phenol content increased in the untreated control plants but rate of increase was insignificant (Table 2). Maximum phenol content was obtained from leaves treated withsalicylic acid 10^{-4} M (4.59 mg/g), statistically at par with $CuSO_4$ 10^{-4} M (4.54 mg/g), followed by $CuSO_4$ 10^{-5} M (3.99 mg/g) irrespective the age of plant. There was no significant difference in increased total phenol content obtained from the plants treated with L-cysteine 10^{-3} M (3.58 mg/g), isonicotinic acid 10^{-4} M (3.59 mg/g), 2, 4 D 10^{-6} M (3.52 mg/g) and sodium selenite 10^{-4} M (3.60 mg/g). Maximum total phenol content was obtained at 96 DAS in all the chemical treatmentsincluding untreated control.

Table 3: Variation in peroxidase content under induced resistance against leaf blight of wheat at different days after sowing (DAS)

Treatments	Peroxidase (µmol/g/min) at different days after sowing					
	40 DAS	54 DAS	68 DAS	82 DAS	96 DAS	Mean
$CUSO_4$ (10^{-4}M)	7.58	12.68	11.82	6.86	5.46	8.88
$CUSO_4$ (10^{-5}M)	8.26	12.72	11.37	8.93	6.09	9.47
$ZnSO_4$ (10^{-4}M)	11.59	10.74	5.95	11.82	8.84	9.79
$ZnSO_4$ (10^{-5}M)	11.14	10.92	7.94	11.86	10.83	10.54
DL-Methionine (10^{-2}M)	8.89	11.82	10.33	10.24	6.86	9.63
DL-Phenylalanine (10^{-3}M)	8.84	12.14	10.33	9.88	6.72	9.58
L-Cysteine (10^{-3}M)	9.83	11.64	10.15	11.28	6.86	9.95
Salicylic acid (10^{-4}M)	5.41	12.81	12.41	6.09	4.24	8.19
Isonicotinic acid (10^{-4}M)	10.87	11.14	10.56	10.65	7.44	10.13
2,4 D (10^{-6})M	11.05	10.96	8.62	11.23	8.53	10.08
Sodium Selenite(10^{-4}M)	10.11	11.59	9.70	10.96	7.13	9.90
Untreated control	12.05	9.79	5.82	12.23	11.05	10.19
MEAN	9.64	11.58	9.58	10.17	7.50	
	SEM (±)			CD at 5%		
Date	0.139			0.389		
Treatment	0.341			0.953		
Date x Treatment	0.482			1.348		

The interaction between the age of the plant and the chemical treatments was also statistically significant in increased total phenol content of the plant. Maximum total phenol content was noticed in plants treated with salicylic acid 10^{-4} M (5.67 mg/g) at 96 DAS, statistically at par with $CuSO_4$ 10^{-4} M (5.66 mg/g).

3.3. Peroxidase activity

The peroxidase activity was dissimilar in plants treated with different chemicals and their level of differences were statistically significant.With the growth of plants the peroxidase activity consistently increases upto 68 DAS. Later on, this trend becomeserratic and decreased peroxidase activity was observed.

The peroxidase activity was observed maximum in plants treated with salicyclic acid (12.41µmol/g/min) followed by $ZnSo_4 10^{-4}$ M (11.82 µmol/g/min). It was statistically at par with$ZnSo_4 10^{-5}$ M (11.37µmol/g/min). In check (untreated control),peroxidase activitywas 5.82 µmol/g/min.It was observed that initially the peroxidase activity was less in all the treatmentsin comparison to untreated control. But peroxidase activity was decreased after 68 DAS to some extend though different inducer chemicals act differently. Finally at 96 DAS all the chemicals significantly reduced the peroxidase activity irrespective of different treatments (Table.3).

Tyagi *et al.,* (1998) reported that the amount of total phenolic and peroxidase activity were higher with increased level of inoculum of *Bipolaris sorokiniana.* Chowdhury *et al.* (2008) observed that biochemical parameters like phenolic, protein, polyphenol oxidase and peroxidase were higher on the resistant genotype than the susceptible one. Beshir (1994) also found that in inoculated plants, phenolic compounds accumulated more rapidly in resistant genotypes than in susceptibleplants. Tyagi *et al.,* (1998) reported that the peroxidase activity was decreased with age in both susceptible and resistant varieties in case of *Bipolaris* infected wheat plants. In current study, was observed that most of the test compounds provided good protection to wheat plant infection by *Bipolaris sorokiniana* by simple seed soaking at extremely low concentration. The bio-chemical treated plants (post-infection) had higher post inflectional total phenol content as compare to untreated one. It appears that in treated plants, there was a rapid accumulation of more phenolic substances at the site of infection within a short period of time. Consequently, reduction in peroxidase and polyphenol enzyme activity. Evidently, increased biosynthesis of phenolic leads to greater accumulation of quinine at the infection site. Vance *et al.*(1980)

reported that enhance lignifications at infection site that reduced the infection frequency by the pathogen.

3.4. Yield and yield attributing characters

Inducer chemicals had significant effecton plant resistance and yield.There was reduction in disease, increasednumber of grains/panicle and 1000 grain weight, which resulted in better yield (Table 4).

3.5. Number of grains/panicle

In the year 2012-13, the Maximum number of grains/panicle was harvested from the plants treated with salicylic acid 10^{-4} M (39).It was statistically at par with $CuSO_4$at two conc. levels (10^{-4} M and 10^{-5} M),and DL-phenylalanine 10^{-3} M (38).Inducer chemicals $CuSO_4$ and its twoconcentrations (10^{-4} M and 10^{-5} M), salicylic acid (10^{-4} M), DL-methionine (10^{-2} M), DL-phenylalanine (10^{-3} M), L-cysteine (10^{-3} M), isonicotinic acid (10^{-4} M), 2, 4-D (10^{-6} M) and sodium selenite (10^{-4} M) showed no significant differences among themselves in respect to no. of grains/panicle. The pooled data showed that seed treatment with salicylic acid (10^{-4} M), $CuSO_4$ (10^{-4} M and 10^{-5} M)and DL-phenylalanine (10^{-3} M) result in maximum no. of grains/panicle. Minimum no. of grains/panicle was noticed in plants treated with $ZnSO_4$ 10^{-4} M (35), statistically at par with untreated control (35).

3.6. The 1000 grain weight

chemicals at different concentration levels increase the 1000 grain weight significantly in comparison to untreated control.The test weight was different in twocopping seasons.The test weight was more in year 2013-14 than year 2012-13. Two years pooled mean showed maximum 1000 grain weight was in salicylic acid 10^{-4} M (47.48 g),statistically at par with $CuSO_4$ 10^{-4} M (46.26 g) seed treatment. Minimum1000 grain weight was obtained in plants treated with$ZnSO_4$ 10^{-4} M (40.72 g). No significant differences in 1000 grain weight was observed among seed treatment with DL-methionine 10^{-2} M (44.33g), DL-phenylalanine 10^{-3} M (44.92 g) and isonicotinic acid 10^{-4} M (44.03 g).

3.7. Total grain yield (q/ha)

Our results showed that chemicals have significant influence on disease reduction in plant, consequently, greater yield of the crop (q/ha). All the chemicals and their different concentration increased the grain yield significantly in comparison to untreated control during both cropping seasons. In the year 2012-13, maximum grain yield was harvested from salicylic acid 10^{-4} M (38.33 q/ha) seed treatment, it wasstatistically at par with $CuSO_4$(10^{-4} M 37.50 q/ha,and 10^{-5} M,36.58 q/ha), DL-phenylalanine 10^{-3} M (36.33 q/ha), isonicotinic acid 10^{-4} M (35.83 q/ha)and sodium selenite 10^{-4} M (35.50 q/ha). In the year 2013-14, maximum yield was harvested from salicylic acid 10^{-4} M (40.00 q/ha). Two years pooled mean data showed similar type of results, that maximum yield was obtained from salicylic acid @ 10^{-4}M (39.17 q/ha) which isstatistically at par with the of $CuSO_4$at its two different concentrations @10^{-4} M and 10^{-5} M (37.92 q/ha and 37.08 q/ha respectively).

Therefore, these inducer chemicals had given good protection to wheat crop against *Bipolaris sorokiniana* by inhibiting the development of this pathogen (Troshina *et al.,* 1991) and also significantly increased the grain yield of wheat (Zaman *et al.,* 2009).Wheat

Table 4: Effect of inducer chemical treatments on yield parameters and grain yield

TREATMENTS	No. of grains/panicle			Test weight (g)			Grain yield (q/ha)		
	2012-13	2013-14	Pooled	2012-13	2013-14	Pooled	2012-13	2013-14	Pooled
$CuSO_4$ (10^{-4}M)	39	40	40	42.67	49.85	46.26	37.50	38.33	37.92
$CuSO_4$ (10^{-5}M)	38	40	39	42.67	48.99	45.83	36.58	37.58	37.08
$ZnSO_4$ (10^{-4}M)	35	36	35	39.20	42.23	40.72	33.17	33.50	33.33
$ZnSO_4$ (10^{-5}M)	35	37	36	39.67	44.20	41.93	32.50	33.90	33.20
DL.Methionine (10^{-2}M)	37	39	38	41.00	47.66	44.33	35.83	36.92	36.38
DL.Phenylalanine (10^{-3}M)	38	39	39	40.67	49.18	44.92	36.33	37.25	36.79
L-Cysteine (10^{-3}M)	35	39	37	40.67	47.27	43.97	35.00	35.75	35.38
Salicylic acid (10^{-4}M)	39	40	40	43.83	51.12	47.48	38.33	40.00	39.17
Isonicotinic acid (10^{-4}M)	36	39	38	40.67	47.39	44.03	35.83	35.08	35.46
2,4 D (10^{-6}M)	35	39	37	40.00	44.60	42.30	34.00	36.25	35.13
Sodium Selenite(10^{-4}M)	37	39	38	40.87	46.98	43.92	35.50	36.58	36.04
Untreated control	34	36	35	38.67	39.55	39.11	29.17	30.83	30.00
Sem (±)	0.64	0.80	0.51	0.64	0.61	0.44	1.12	0.88	0.71
CD (0.05%)	1.89	2.35	1.47	1.87	1.78	1.26	3.28	2.59	2.03

plant infected by biotic agents or treated with abiotic agents may resulted in the local or systemic induction of disease resistance against leaf rust pathogen attack both at seedling and adult plant stages (Tahamey and EL-Sharkawy, 2014).

Based on our results, it is suggested that seed soaking with inducer chemical compound like salicylic acid (10^{-5} M),$CuSO_4$(10^{-4} M and 10^{-5} M) could be used in the management of spot blotch of wheat caused by *Bipolaris sorokiniana*. These compounds also increased the grain yield of wheat.However,lengthening the effect of these inducers on disease resistance, requires one more foliar spray at boot leaf stage of the crop.

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