

Evaluation of Efficacy of Different Fungicides to Manage Stemphylium Leaf Blight Disease in Lentil

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ABSTRACT

Stemphylium blight (*Stemphylium botryosum*) is becoming a major disease of lentil in recent years that results in large scale defoliation and its severity is increasing gradually in North Eastern plain zones of India. An experimental trial was conducted to check the efficacy of different fungicides and were tested *in-vitro* and *in-vivo* against *Stemphylium botryosum*. Among the six test fungicides viz., Saaf (Carbendazim 12%+ mancozeb 63% WP), Krilaxyl (Metalaxyl 8%+ Mancozeb 64% WP), Amistar (Azoxystrobin 23% W/W), Nativo (Tebuconazole 50% +Trifloxystrobin 25% WG), Contaf (Hexaconazole 5% SC) and Delma (Azoxystrobin 8.3%+ Mancozeb 66.7% WP), Contaf, Nativo and Delma showed higher growth inhibition of *Stemphylium botryosum* under *in-vitro*

condition. Under *in vivo* field conditions, lowest diseases severity and highest disease reduction was found with the spray treatment of Delma followed by Amistar and Contaf. and percent yield increase was highest in Delma treated plot followed by Contaf as compared to control.

Keywords Stemphylium, Lentil, Efficacy, Fungicides.

INTRODUCTION

Lentil, rainfed *rabi* crop, is the second most important legume crop next to chickpea in India which serves as an inexpensive source of high- quality protein with low fat and high fiber content ensuring nutritional security mainly in the rural population of India. It was cultivated in 52 countries on 30.5 million ha area with an annual production of 19.9 million tones and had a productivity of 654.44 kg/ha in 2014 where India is the second largest producer next to Canada (FAO 2016). Production of lentil in India is concentrated in eastern Uttar Pradesh (UP), Chhattisgarh, Bihar and West Bengal where Madhya Pradesh ranks first in acreage i.e., 39.89% (5.96 lakh ha) followed by UP 31.99% (4.78 lakh ha) and Bihar 10.03% (1.50 lakh ha) and in terms of production UP ranks first with 45.09% (6.79 lakh tones) followed by Madhya Pradesh (29.69%) and Bihar (9.47%) in terms of production. Concerted efforts are already

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been taken by the Govt of West Bengal to increase the area under pulses with major emphasis on lentil with present area of 1 lakh ha contributing 6.16% of the national production (“Pulses Revolution- From Food to Nutritional Security”, Ministry of Agriculture, GOI). However, the yield of lentil is not very encouraging in India because of the occurrence of various biotic and abiotic stress factors at different growth stages (Mandal *et al.* 2019). Other reasons include growing of nondescript, farmer-preserved lentil varieties mainly on tiny land holdings with marginal soils, under rainfed conditions and insignificant adoption of improved varieties, poor agronomic practices, management of nutrients and inability to deal with diseases also cause significant yield losses (FAOSTAT Database 2020). Lentil is reported to be suffered from many diseases like Botrytis grey mold, Anthracnose, Ascochyta blight, Stemphylium blight, wilt, root rot. which reduce the productivity of lentil by 20–25% (Sharma and Shukla 2014). Among these diseases, Stemphylium blight caused by *Stemphylium botryosum* Wallr. (Once a minor disease) is becoming a serious threat in recent years (Mwakutuya and Banniza 2010) and also causing major constraints to the farmers mostly on northern parts of West Bengal (Das *et al.* 2017 and Mondal *et al.* 2017) due to its climatic condition as the part of the state falls under humid tropic region of the India (Kant *et al.* 2017). In India, the disease was recorded by Nene *et al.* (1984) and the intensity of the disease was 82.55% and the loss was recorded as 93.4% (Singh *et al.* 1999). Therefore, disease management is essential among which use of fungicide is the most dependable method of control of plant diseases in a successful and economical perspective. So far, the evaluation of efficacy of different fungicides is nowhere properly recorded for control of Stemphylium blight on lentils. Furthermore, no fungicide with current/ anticipated registration on lentil has been tested against Stemphylium blight on lentils (Wunsch 2013). In this experiment some commonly used fungicides in West Bengal state were tested (both *in vitro* and *in vivo*) to check their efficacy against the disease.

MATERIALS AND METHODS

The field experiment was carried out at the in-

structional research farm of the university and the laboratory related works were done at the Research Laboratory, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal.

Isolation and purification of fungal pathogen

The spots/ lesions formed on the diseased leaves were selected and cut into small pieces, surface sterilized in mercuric chloride solution (1:1000) for 35-40 minutes in a petri-plate and were washed 3-4 times with sterilized water. The cut pieces were inoculated in sterilized petriplates containing potato dextrose agar (PDA) medium and kept in BOD at $25\pm 1^\circ\text{C}$ for the isolation and growth of the causal pathogen. Then isolated fungal culture were purified and maintained in culture tube to avoid contamination.

Evaluation of *in vitro* inhibitory potential of the fungicides against *S. botryosum*

Six different fungicides were studied and evaluated under *in vitro* condition against *S. botryosum* using poisoned food technique. The six different molecules or combinations of fungicide molecules in different concentrations used were namely Saaf (Carbendazim 12%+ Mancozeb 63% WP), Krilaxyl (Metalaxyl 8%+ Mancozeb 64% WP), Amistar (Azoxystrobin 23% W/W), Nativo (Tebuconazole 50% + Trifloxystrobin 25% WG), Contaf (Hexaconazole 5% SC) and Delma (Azoxystrobin 8.3% +Mancozeb 66.7% WP). Four fungicides viz, Saaf, Krilaxyl, Amistar, Nativo were taken at the concentrations of 100 ppm, 200 ppm, 400 ppm, 800 ppm, 1600 ppm for each fungicide and two fungicides Contaf and Delma at the concentrations of 25 ppm, 50 ppm, 100 ppm, 200ppm and 400 ppm (for each fungicide) were taken for the study purpose. For each fungicide and control, three replications were prepared where control was maintained without any fungicide. Growth in all concentrations was taken and % inhibition over control as well as ED50 values for the treatments was found out. In case of liquid fungicides (Contaf and Amistar) 8 ml each were taken and mixed with 200 ml of sterilized distilled water while dust form fungicides (Saaf, krilaxyl and Delma) and granular form fungicide (Nativo), 4 g each is mixed in a conical flask with the 200 ml distilled water.

Mycelial discs of 15- 20 days old culture were placed at the center of the petri plates and then incubated at $25\pm 1^\circ\text{C}$ in BOD incubator for mycelial growth. The diameter of the fungal growth in every petriplates of different concentration was recorded in every 4th day till the full growth in the control plate.

Field/ *in-vivo* trial

Field trial were carried out at the instructional research farm of the university on the susceptible lentil variety WBL77 and was sowed in a plot size of $2\text{m} \times 1\text{m}$ based on Randomized Block Design on 6th Dec, 2017 with three replications. All the cultural practices like fertilizer application, weeding and irrigation were done based on recommended applications. The six fungicides under study were taken for chemical application as foliar spray (2 g for dust and granular fungicides and 1 ml for liquid fungicides in 1 liter of water). Total three (03) spraying of the same fungicides namely Saaf, Contaf, Krilaxyl, Delma, Amistar and Nativo were done at 10-15 days interval. The disease severity was recorded at regular time interval starting from disease appearance in field as PDI (Percent Disease Index) using (Hashemi *et al.* 2005). The control plot was maintained without any treatment. And the data of fungicidal evaluation (both *in vitro* and field) or data analysis was done with the Microsoft Office Excel RBD and CRD Training software.

RESULTS AND DISCUSSIONS

The results obtained from the experiment done to evaluate *in-vitro* efficacy of different fungicides in controlling *Stemphylium* blight of lentil has been presented in Table 1. From the study, it was observed that among all the fungicides tested, Contaf (Hexaconazole 5% SC) showed the lowest ED_{50} value of 50 ppm which is followed by fungicide trade name Delma (Azoxystrobin 8.3%+ Mancozeb 66.7% WP) with a value of 128 ppm, Krilaxyl (637 ppm) and Saaf (1028 ppm). The data also indicates the percent inhibition in growth of test fungus by different fungicides at different concentrations and it was found that fungicide Contaf, Nativo and Delma showed highest reduction in growth with 83.87%, 81.91% and 72.04 % respectively at 400 ppm. Nevertheless surprisingly, among all fungicides tested, Nativo gave

best result in percent inhibition (79.79 %) even at 100 ppm while Saaf and Krilaxyl reduced the fungal growth by 58.18% and 65.29 % respectively, at 1600 ppm. Contrary to this, no considerable reduction percentage between the highest and lowest dose was found in case of Amistar and Nativo. Under *in-vitro* condition, Amistar showed very less efficiency to control the growth of the *Stemphylium botryosum*. After a careful scrutiny of literature, it was found that different researchers also have conducted various similar experiments and out of which, some results pertaining to the similar findings are in Hosen *et al.* (2009) and Rahman *et al.* (2010) where they reported the percent inhibition of *S. botryosum* in plates treated with 2000 ppm Agromil 72WP (Metalaxyl + Mancozeb) as 61.77% and 63.19 % respectively. In the year 2014, Subedi and his co- workers also used different fungicides to check the efficacy against *S. botryosum* at different concentrations (500 ppm, 1000 ppm, 1500 ppm and 2000 ppm) and found that the mycelial growth inhibition percent of Saaf, Mancozeb and Bavistin @ 2000 ppm were shown as 68.79, 55.94 and 47.18 respectively. The relation between fungicide concentration and percent growth inhibition of a specific fungus was drawn by taking log value of fungicide concentration and probit value of percent growth inhibition. The linear regression lines with equations were obtained for each of the selected fungicides against the test fungus and presented graphically in the Fig.1. The trend of regression lines as depicted in Fig.1. indicate that there is increase in growth inhibition percentage with the increase in fungicide concentrations in case of all the test fungicides. Furthermore, a positive correlation between fungicide

Table 1. Growth inhibition of *Stemphylium botryosum* by fungicides. *ND = Not Detectable.

Fungicide	Dose (ppm)					R ²	ED50
	100	200	400	800	1600		
Saaf	21.53	28.99	34.58	48.03	58.18	0.604	1029
Krilaxyl	19.13	23.08	42.80	58.97	65.29	0.9618	637
Amistar	10.34	10.74	13.72	15.71	17.10	0.9629	ND
Nativo	79.79	81.06	81.91	82.98	83.40	0.9262	ND

Fungicide	Dose (ppm)					R ²	ED50
	25	50	100	200	400		
Contaf	37.85	51.61	60.22	72.04	83.87	0.988	50
Delma	21.43	38.49	45.44	53.37	73.02	0.96	128

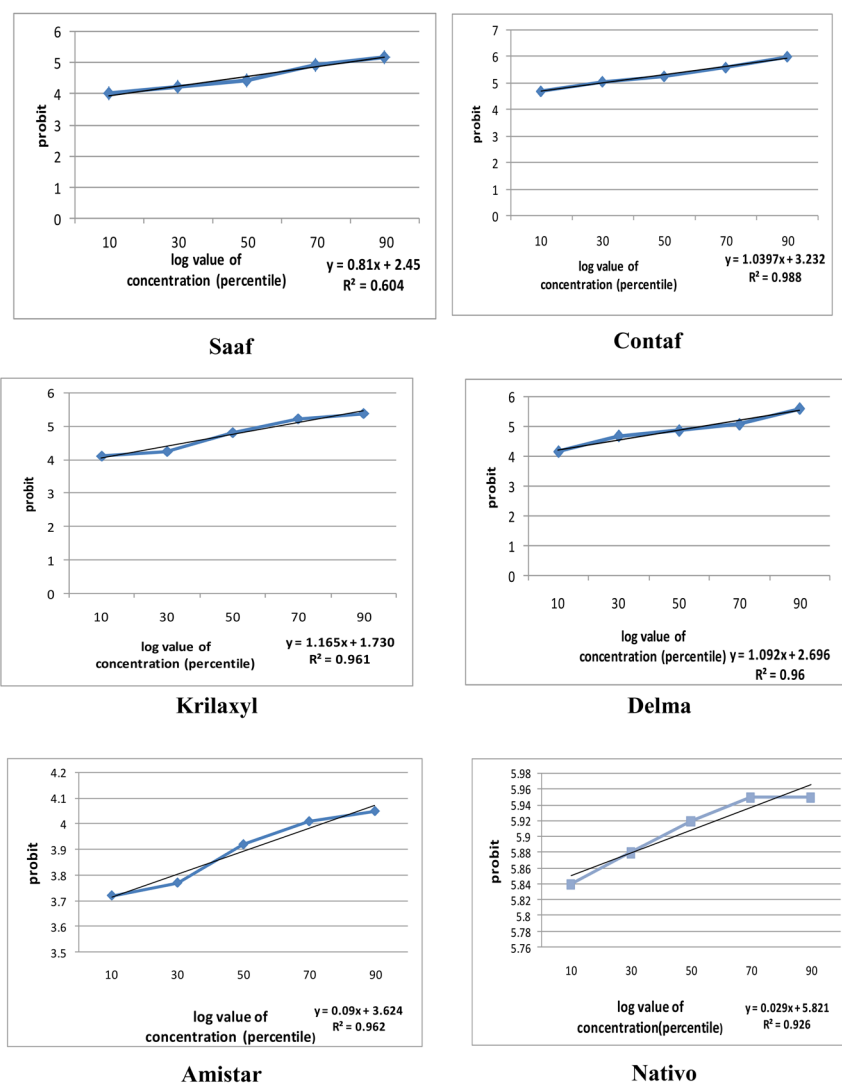


Fig. 1. Regression between fungicide concentration (log) and growth inhibition percentage (Probit).

concentration and growth inhibition percentage was found in all the cases. Therefore, the ED_{50} values for each of the fungicides were calculated from regression equations as shown in the figure and presented earlier in Table 1.

The result of the *in vivo* (field) experiment is presented in Table 2. From the results shown in Table 2, all the fungicidal treatments were found to give significantly lower disease severity than control. This is also evident from observation (taken after spray)

that lowest diseases severity was obtained in case of Delma with a PDI of 3.24 % which is followed by Amistar and Contaf with disease severity of 3.43% and 4.74 %, respectively. Additionally, the maximum disease reduction was found in Delma (92.27%) as compared to check or control plot. Among the other treatments observed, good results were obtained by Amistar (91.80%) and Contaf (88.70 %) while Percent Disease Control (PDC) of the Krilaxyl and Saaf is 41.15% and 14.01%, respectively. Moreover, the PDI was obtained from the plot treated with Saaf

Table 2. Effect of fungicides on *Stemphylium* blight severity and yield.

Fungicides	PDI		Percent Disease control	Percent yield increase
	Before spray	After spray		
Saaf	4.14	36.06	14.01	35
Contaf	3.44	4.74	88.7	82
Krilaxyl	3.89	24.68	41.15	61.5
Delma	1.58	3.24	92.27	93.5
Amistar	2.12	3.44	91.8	78.5
Nativo	3.72	34.86	16.89	39.5
Control	4.88	41.94		
SEm	0.07	3.71		
SEd	0.09	5.25		
CD (5%)	0.217	11.45		
CD (1%)	0.305	16.05		

(36.06%), Contaf (4.74%), Krilaxyl (24.68%), Delma (3.24%), Amistar (3.43%), Nativo (34.86%) comparison to control value. It is clearly evident from the study that Delma is showed lowest disease severity which is closely followed by Amistar and Contaf. The higher percent yield increase was obtained from Delma treated plot (93%) followed by Contaf (82%) over control. In the plots treated with Saaf, Krilaxyl and Nativo 35%, 61.50 % and 39.50% increase in yield was found, respectively.

An experiment was also conducted by Rahman and his co-workers in the year 2010 using fungicides namely Iprosun 50 WP (Iprodione), Eduzeb 80WP (Mancozeb 80WP), Proud 25EC (Propiconazole), Rovral 50WP (Iprodione), Agrimyl (Mancozeb+Metalaxyl) @ 0.2% in field condition to evaluate the efficacy of fungicides and reported that Iprosun 50 WP gave the highest yield increase (90.78%) and lowest disease severity over the control. In 2015, Shahiduzzaman and his co-workers also carried out similar field experiment to evaluate the efficacy of five fungicides against *Stemphylium* blight (*S. botryosum*) of lentil during *rabi* seasons of 2011-12 and 2012-13 in which the fungicides tested in the experiment were Rovral 50WP (Iprodione), Secure 600 WG (Fenamidione+ Mancozeb), Companion, Indofil-45 (Mancozeb) and Nativo 75WG (Tebuconazole+ Trifloxystobin) were applied as foliar spray at 0.2% suspension in plain water. Inoculation potential was 106. Results from this experiment stated that highest severity of *Stemphylium* blight was found under control, statistically

Table 3. Related yield and relative economics of various fungicidal treatments.

	Yield (kg/ha)	Relative cost (Rs/ha)	Relative yield (kg)	Relative return (Rs/ ha)	Relative net Profit (Rs/ ha)
Control	222	0	0	0	0
Saaf	300	1080	78	5460	4380
Contaf	404	270	182	12740	12470
Krilaxyl	359	1632	137	9590	7958
Delma	430	2520	208	14560	12040
Amister	397	2364	175	12250	9886
Nativo	310	9768	88	6160	-3608

similar to the treatments with Nativo and Companion and other fungicides Rovral followed by Secure as well as Indofil significantly reduce disease severity over control. Though all fungicides increased plant height and pod yield per plant compared to control. Significant increase in plant height and pod number was achieved with only Nativo, Secure and Rovral.

Results from the Fig. 2, a positive correlation was found between Percent Disease Control and Percent Yield increase in the experiment of different fungicides against *Stemphylium* blight as shown by the equation, $y = 1.539x - 42.61$ and coefficient of regression (R^2) = 0.941. Therefore, it is clear that with the increase in the percentage of disease control there is increase in yield. The relative net profit calculated and presented in Table 3, for the fungicidal trial under the field condition against *Stemphylium* blight disease of Lentil. It was found that Contaf and Delma are the most effective and also the most economic fungicides in the management of *Stemphylium* blight of Lentil which is followed by Amistar.

CONCLUSION

Under *in-vitro* evaluation, Contaf, Nativo and Delma showed higher growth inhibition of *Stemphylium botryosum* among the fungicides used in the research experiment. The ED_{50} values for each of the fungicides were calculated from the regression equations which again indicate Contaf is having minimum ED_{50} value followed by Delma against the test fungus. Amistar is not showing much effect or efficacy under *in-vitro* condition. Thus, it may be concluded that in the field evaluation of fungicides against *Stemphylium* blight

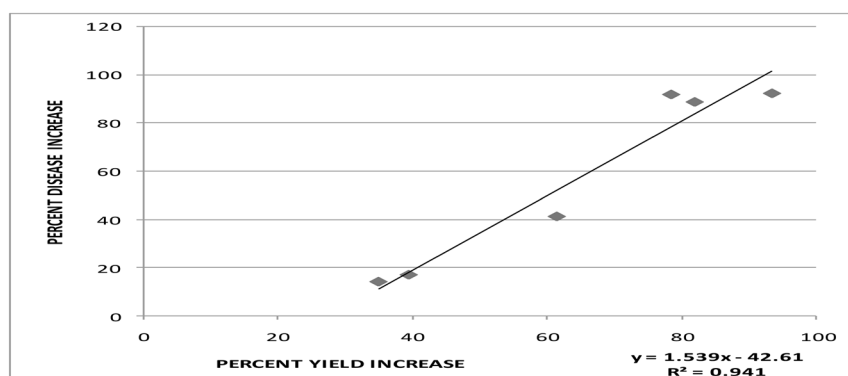


Fig. 2. Relation between percent disease control and percent yield increase.

of Lentil lowest diseases severity and highest disease reduction was found with the spray treatment of Delma followed by Amistar and Contaf. Percent yield increase was highest in Delma treated plot followed by Contaf as compared to check, among the test fungicides. Furthermore, these two fungicides also found economically sound to manage *Stemphylium* blight of lentil and may be recommended to farmers for managing the disease under field condition to achieve higher disease control as well as higher yield. Further studies may be conducted to find out the integrated way of managing the disease as well as on recent novel/ techniques for detection and management of pathogen causing the disease.

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