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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2648-2650

www.thepharmajournal.com
Received: 06-07-2021
Accepted: 16-09-2021

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Impact of crude toxin on radicle and plumule length

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Abstract
Leaf blight of wheat (Bread wheat - *Triticum aestivum*) is mainly caused by *Helminthosporium sativum* (Pammel, King & Bakke) in India and neighbouring south asia countries and capable of causing losses in yield up to 50% in susceptible variety as well as results poor grain quality. With a view to find out effect of toxin produced by different isolates of *H. sativum* (H_1, H_2, H_3, H_4 and H_5), their crude toxin were evaluated against germination of wheat seed of cultivar UP 262 at dilutions 1:20, 1:100, 1:200, 1:500 and 1:1000. The observation recorded after 96 hours, showed the dilution 1:20 did not allow seed germination however, 1:100 showed germination which was significantly lower as compared to other dilutions. The observation recorded after 48 and 96 hours of seed treatment showed that the radical and plumule length was drastically reduced in some of the dilutions of crude toxin of all the isolates as compared to check. This study facilitate to screen a number of variety against diasese (resistant/susceptible) within a short period of time.

Keywords: Crude, toxin, radicle, plumule, length

Introduction
Wheat globally occupies 217 million hectare area among all crops with an annual production around 731 million tones reported by USDA 2018. Wheat is one of the most cereal crop grown worldwide and liking staples of nearly 2.5 billion of world population. India is the second largest producer of wheat worldwide (29.58mh and global area 14% and production 99.70mt.) reported by Sharma and Sendhil (2015 and 2016) [7, 8]. The wheat suffers a number of diseases caused by bacteria, fungi nematodes and viruses. Among fungal diseases, leaf blight of wheat (caused by *Helminthosporium sativum*) is most important and noticed in India as early as in 1924 (Kulkarni 1924) [5]. The *H. sativum* produces toxin helminthosporal which have effect on radicle and plumule length. The study of consist of evaluation of toxicity of different isolates of *Helminthosporium sativum.* Joshi *et al*. 2007 [4] reported that this is very serious pathogen in warm humid region of the world. Toxin play an important role in etiology of the leaf spot disease. There are few earlier reports on phytotoxic production which are sesquiterpenes and non host specific. For this study an attempt was made to extract purify assay and characterized toxin in the culture filtrates of pathogenic isolates (H_1, H_2, H_3, H_4 and H_5) on variety UP-262 collected from G.B. Pant University of agric.& tech, pantnagar and N.D. University of Agric.& Tech. Kumarganj, Faizabad.

Materials and Methods
The fungi were grown in one Litre Erlenmayer flask Containing 200 ml P.D. broth medium 28 days in still culture at 25 to 28 ^{0}C and filtrate was obtained by filtration through two layer of muslin cloth to separate mycelium. This filtrate was known as culture filtrate. The culture filtrate was then filtrated true whatman filter No.-1 to remove most of the mycelium fragment and spores.

Partial purification of culture filtrated
For this 250ml culture filtrated was taken in 1000ml separating funnel. To that 250ml of solvent (90% chloroform + 10% ethyl acetate) was added. The whole content was shaken vigorously and then kept undisturbed on stand. Two layer were formed the upper aqueous phase and the lower chloroform phase. The lower phase was collected in a 500ml beaker. The same procedure was repeated thrice. To the beaker 100g.of anhydrous Na_2SO_4 was added to break emulsion. The steered all was filtrate through whatman No.-1 filter paper and residues was discarded. The liquid in filtrated was completely evaporated at 38^{0}C in vacuum. The browny residues dissolved in ethyl alcohol, through shaken and collected in test tube. This was use as crude toxin or partially purified toxin.

Result and Discussion

Effect of partially purified toxins of *H. sativum* at five dilution i.e (1:20, 1:100, 1:200, 1:500 and 1:1000) on seed germination was studied. The result are presented in Table-1.

Table 1: Effect of crude toxins of *H. sativum* on seed germination (after 96 hours)

Dilutions	Isolates					
	H_1	H_2	H_3	H_4	H_5	Mean
1:20	0.00	0.00	0.00	0.00	0.00	0.00
1:100	39.03	38.39	36.36	37.58	35.17	37.30
1:200	74.20	73.69	71.64	70.20	67.16	71.37
1:500	92.85	90.24	90.15	87.34	84.83	88.68
1:1000	100.00	99.00	100.00	100.00	10000	99.80
Check	100.00	100.00	100.00	100.00	100.00	100.00
Mean	67.68	66.88	66.35	65.85	64.52	66.19

CD_1 at 5% (Isolates) 1.97, CD_2 at 5% (dilution) 2.16
CD_3 at 5% (IxD) 4.83 CV 4.49

The least germination (64.52 percent) was recorded in partially purified toxin of isolate H_5 and maximum (67.68 percent) in H_1 when seeds were placed on toxin soaked blotter. Comparison of average seed germination indicated that toxins of all the isolates showed non-significant difference among themselves in inhibiting seed germination.

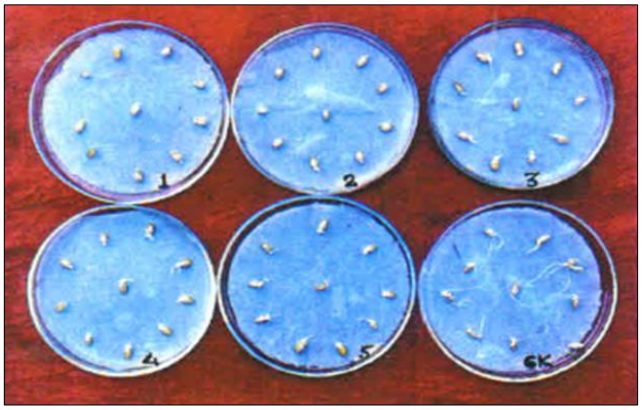

Fig 1: Effect of crude toxin on seed germination

1: 20 Dilution, 1: 100, 1: 200, 1: 500, 1: 1000, Check

At different dilutions, germination ranging from 0.00 to 100 per cent was recorded Dilution 1:20 of all the isolates did not allow seed germination, however, dilution 1:100 showed germination which was significantly lower as compared to the other dilutions. Dilution 1:1000 showed maximum (99.8 percent) seed germination was good as check. All the dilutions different significantly among themselves in recording seed germination.

Effect of toxin on radicle length

The crude toxins of different isolates of *H. sativum* was evaluate for is effect on the growing radicle of the germinated seed after 48 hours and 96 hours of treatment. The minimum radicle length of 6.8mm was recorded in isolate H_5 and maximum (8.13mm) in H_1 after 48 hours of treatment. All the isolates had non-significant differences among themselves in reducing radicle length.

In crude toxin dilutions of 1.20 and 1.100 there was no germinations recorded whereas, other dilutions showed germination given in table 2a. Comparison among dilutions showed the minimum radicle length (7.02mm) in dilution 1:200 and the maximum (12.42mm) in 1:100 which were significantly lower then check. All the dilutions showed significant differences among themselves in inhibiting radicle growth.

Table 2a: Effect of crude toxins of *H. sativum* isolates on radicle length (mm) after 48 hours of seed treatment

Dilutions	Isolates					
	H_1	H_2	H_3	H_4	H_5	Mean
1:20	0.00	0.00	0.00	0.00	0.00	0.00
1:100	0.00	0.00	0.00	0.00	0.00	0.00
1:200	9.40	8.67	10.22	8.16	6.70	7.02
1:500	11.32	10.30	11.24	10.22	10.20	10.54
1:1000	13.63	12.24	11.93	12.22	10.12	12.42
Check	1447	15.32	13.25	14.96	12.95	14.65
Mean	8.13	7.75	7.77	7.59	6.80	7.43

CD_1 at 5% (Isolates) 1.24, CD_2 at 5% (Dilution) 1.35
CD_3 at 5% (IxD) 3.03, CV 10.20

Table 2b: Effect of crude toxins of *H. sativum* isolates on radicle length (mm) after 96 hours of seed treatment

Dilutions	Isolates					
	H_1	H_2	H_3	H_4	H_5	Mean
1:20	0.00	0.00	0.00	0.00	0.00	0.00
1:100	10.03	7.64	9.07	8.46	7.63	8.56
1:200	22.04	18.91	22.99	20.05	20.02	20.40
1:500	32.14	28.46	29.13	30.40	29.31	29.88
1:1000	36.33	35.60	33.35	34.55	32.53	34.47
Check	38.07	35.42	35.61	35.22	35.90	36.04
Mean	23.10	21.00	21.36	21.44	20.90	21.56

CD_1 at 5% (Isolates) 1.63, CD_2 at 5% (Dilution) 1.78
CD_3 at 5% (IxD) 4.02, CV 9.80

The observation recorded after 96 hours of the treatment of the seeds showed that the isolate H_5 allowed minimum radicle length (20.9) Whereas isolate H_1 recorded maximum (23.10mm) radicle length. All the isolates had non-significant differences among themselves.

At the dilution of 1:20 radicle did not emerge, whereas, in other dilutions radicle emerged and the growth of radicle was recorded.

The radicle length at all the dilutions showed significant difference than check, whereas dilution 1:1000 was at par with check. The least radicle length (8.56) was found in dilution 1:1000 (table 2b).

Effect of toxin on plumule length

Effect of toxins on plumule length of the germinated seeds was also recorded after 48 and 96 hours. The plumule length was also minimum (3.46mm) in isolate H_5 and maximum (4.26mm) in H_1, All the isolates showed non-significant difference among themselves with respect to plumule length.

At the dilutions of 1:20 and 1:100 plumule did not emerge where in other dilutions plumule length was recorded, given in table 3a the minimum plumule length of 5.7 mm was found at dilution 1:200 and maximum 6.24mm and dilution 1:100 the dilution 1:200 had non-significant difference with 1:500 however it was significantly more inhibitory then dilution 1:100.

Then dilutions among themselves did not show any difference inducing plumule length the observation taken after 96 hours of treatment also indicated that isolate h_5 had minimum (12.51mm) plumule length and isolate H1 the maximum (13.66 mm) All the isolates showed non-significant differences among themselves.

Table 3a: Effect of crude toxins of *H. sativum* isolates on plumule length (mm) after 48 hours of seed treatment

Dilutions	Isolates					
	H_1	H_2	H_3	H_4	H_5	Mean
1:20	0.00	0.00	0.00	0.00	0.00	0.00
1:100	0.00	0.00	0.00	0.00	0.00	0.00
1:200	5.31	4.03	5.16	4.36	4.23	4.67
1:500	6.39	604	5.99	5.51	5.26	5.83
1:1000	6.60	6.21	666	6.28	5.47	6.24
Check	7.26	7.02	7.52	7.31	5.81	6.98
Mean	4.26	3.86	4.22	4.22	3.46	3.95

CD_1 at 5% (Isolates) 1.20, CD_2 at 5% (dilution) 1.31
CD_3 at 3% (IxD) 2.94, CV 8.50

Table 3b: Effect of crude toxins of *H. sativum* isolates on plumule length (mm) after 96 hours of seed treatment

Dilutions	Isolates					
	H_1	H_2	H_3	H_4	H_5	Mean
1:20	0.00	0.00	0.00	0.00	0.00	0.00
1:100	5.21	5.47	4.71	4.93	6.43	5.35
1:200	10.73	9.57	9.68	10.05	8.75	9.75
1:500	19.38	18.72	19.20	21.21	16.75	18.85
1:1000	22.60	22.26	21.46	21.74	21.01	21.81
Check	24.05	23.06	23.98	22.37	22.15	23.13
Mean	13.66	13.18	13.18	13.21	12.51	13.15

CD_1 at 5% (Isolates) 1.22, CD_2 at 5% (dilution) 1.34
CD_3 at 5% (IxD) 3.00, CV 9.8

Comparison of average growth of plumule in different dilutions of isolates suggested that dilution 1:20 was completely inhibitory to germination, therefore, plumule emergence but toxins at other dilutions allowed plutonic to emerge from the treated seeds showed in Table 3b. Out of these dilutions, the dilution 1:20 allowed minimum plumule length of 5.35 mm and 1:100 allowed maximum (21.8Imm) which was at par to untreated check. All the dilutions showed the growth of plumule was significantly lower than check and they had significant differences among themselves.

Application of a drop of spore suspension of pathogen, crude toxin, TLC purified toxin and distilled water (check) separately on detached wheat leaves in *in-vivo* condition, showed different size of lesions produced by different isolates. It was revealed from the photograph that toxin preparation having culture filtrates of the pathogens by precipitation and extraction with organic solvent (chloroform 90% + ethyl acetate 10%) and evaporating liquid completely through flash evaporator and the residues dissolved in absolute alcohol was used as crude toxin.

Ghislaine and Closset (1978) [1] also reported similar results in inhibition of germination of seeds barley variety Gypsy. Cpari and Marysa of wheat the culture filtrate of *H. sativum.* Pegg (1976) [6] suggested that the effect of indole acetic acid (IAA), a growth regulation substance, could be attributed in causing root inhibition. Inhibition of elongation of radicle and plumule in pigeonpea, similar to that by culture of *Fusarium udum*, by naphthalene acetic acid was proved by Singh (1986) [9], Jahani *et al.* (2006) [2] and Jahani *et al*. 2014 [3].

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