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Herbicides Impact on the Growth of *Alternaria alternata* and Morphological Variation in Spores under *in vitro* Condition

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Abstract: ***Herbicides impact was assessed against phytopathogenic fungi Alternaria alternata using different concentrations on the colony growth, spores structure, spores germination and elongation of germ tube. At the 5000 ppm concentration growth of A. alternata was completely restricted but some growth was found in fluchloralin and benthiocarb treated samples at the same concentration. At low concentration (10 ppm) stimulation in growth was occurred in 2,4-D treated Petri plates. Herbicides were found to promote abnormalities in the spores structures at higher concentration. Spores of A. alternata growing on untreated PDA had mean length 27.5 um width 9.5 um; number of horizontal septa 5.0 and vertical septa were 3.0 and 2.0 respectively. At 5000 ppm concentration in 2,4-D treated medium sporulation was prohibited and colour of colony became white. Herbicides also changed the spore structure. In 2,4-D treated cultures mean length of spore decreased by 3.27 um and mean width increased by 1.80 um. Number of horizontal septa also decreased but number of vertical septa was not affected. In case of benthiocarb, spore length decreased considerably while no marked change in width was found. In fluchloralin mean length of spore was 17.12 um and mean width 12.65um. In benthiocarb and fluchloralin treated culture the mean number of horizontal septa were 1.0 and no vertical septa was observed. Percentage germination of spores and growth of germ tube was also affected by the herbicides and inhibitory effect was corresponding to the concentration of herbicides.***

Keywords: Herbicide, 2,4-D, fluchloralin, benthiocarb, fungi, *Alternaria alternata*

1. Introduction

Weeds are found to grown with the cultivated crops. Existence of the weeds with cultivated crops are found to reduce the yield of crops by providing completion of soil nutrients as well as the available diminishing the available space for development. To eradicate the weeds fro cultivated crops herbicides are used as a chemical substance. In recent past year's use of herbicides has become in immense practice. Herbicides which are used to remove the weeds from the agricultural field might have an impact on the fungi either associated with phyllosphere of the cultivated crop plants, rhizospheric region of the plants and also the soil. As such these fungi are non target organisms. To be effective, herbicides they must have strong biological activity against plants. Indirectly through their effects on plants, herbicides can influence almost any process or interaction of the plant, including its susceptibility to plant diseases. In some cases, herbicides also have direct effects on plant pathogens. Application of herbicides in field may have an influence on the fungal population associated with phyllospheric region. Effect may be both increase and decrease of population or change in the physiology as well as morphological structures. On the other hand phytopathogenic fungi are also found to be associated with phyllospheric region and similarly they may be affected by application of herbicides. Many studies have not been undertaken on the impact of herbicides on the phytopathogenic fungi.

Alternaria alternata (Fr.) Keissler is one of the causes of brown leaf spot disease of potato, it generally effects on the above ground part causing a serious loss in the yield. *Alternaria alternata* has been reported as a leaf pathogen on a number of other hosts which include crops and trees of immense economic value. *A. alternata* has several pathotypes which cause number of diseases in other plants such as; stem canker of tomato (Grogan *et al*., 1975), *Alternaria* black spot of strawberry (Tsuge *et al*., 2011), leaf spot and fruit rot of chilli (Ginoya and Gohel, 2015), leaf spot of bean, black point of wheat (Cromey and Mulholland, 1988), stem rot of mango (Li *et al*.,2018), and *Alternaria* fruit rot of persimmon (Prusky *et al*., 1981). Early blight can cause yield losses of 5- 50 % (Tsedalye, 2014), and in some continents it reaches up to 73 % (Kapsa, 2009). Early studies conducted by Tsedalye, (2014) revealed that among many diseases of potato, foliar diseases (i.e. early blight and/or brown spot) are the most important and destructive diseases worldwide, particularly in areas with favorable weather conditions, where it causes reduction in quantity and quality of this crop.

Considering the above mentioned aspects study was carried out to determine the influence of herbicides on the phytopathogenic fungi *A. alternata*.

2. Materials and Methods

Alternaria alternata was procured from Indian Agricultural Research Institute, New Delhi. Further pure culture was maintained on Potato Dextrose Agar medium (PDA).

Fungal growth measurement as linear extension on Agar medium

To evaluate the efficacy three commonly used herbicides were taken for study viz., (a) Benthicarb or Saturn (S-(4 chlorabenzyl)-N, N diethyl thiol carbamate) (b) 2,4-D or weedon (2,4-Dichloro phenoxy acetic acid) (c) Fluchloralin or Basalin (N-propyly-N(2' chloroethyl)-2, 6 dinitro-n-

trifluromethyl aniline). A series of herbicides concentrations viz.; 0, 10, 100, 500, 1000, and 5000 ppm were prepared by incorporating them in PDA medium. 20 ml of each control and treated PDA was dispersed into Petri plates having 90 mm diameter. Five replicates were maintained for each concentration and control. About 3 hours after the plats were poured; a mycelia disc of 5 mm diameter taken from the periphery of a 5 days old culture of *A. alternata* growing on PDA was transferred to the centre of each plate. The plates were incubated at 25°C and fungal growth rate was determined by measuring the diameter of colony at 24 h intervals. Two measurements at right angle to one another where made each time. Measurements were continued until the fungus had grown up to the periphery of the plate or for a maximum of 7 days.

Spores germination and germ tube elongation

Herbicides solutions were prepared in distilled water at the concentration of 10, 100, 500, 1000 and 5000 ppm. The spores taken from a 5 days old culture were suspended in the above fungicides solutions. From each of the suspension a hanging drop was made on a cavity slide. The cavities we are sealed with Vaseline. Five slides were prepared for each treatment. Slides were incubated in dark at 25°C in dark. Observations were taken at the interval of 24, 48 and 72 hours. Spores germination and germ tube growth was observed in 10 randomly chosen platforms from each of the slides at a micro scope projection of 10 x 10.

3. Results

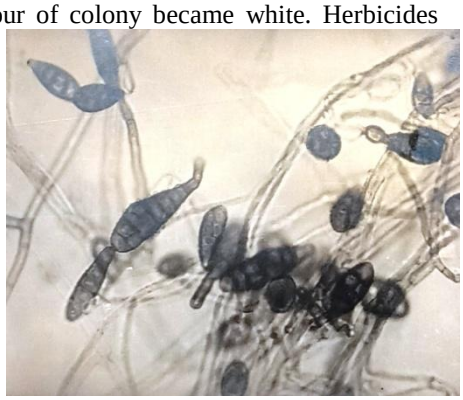
Fungal colony growth and morphological variation in spores

Effect on the growth of colony of herbicides treated plates was slower than the control. At the 5000ppm the growth was totally checked in case of 2,4-D, however on the fluchloralin and benthiocarb treated plates some growth was recorded (Table1). At low concentrations of herbicides growth of fungi in case of 2,4-D treated plates was almost similar to control. But in case of fluchloralin and benthiocarb even at low concentrations growth was always remain lower than control.

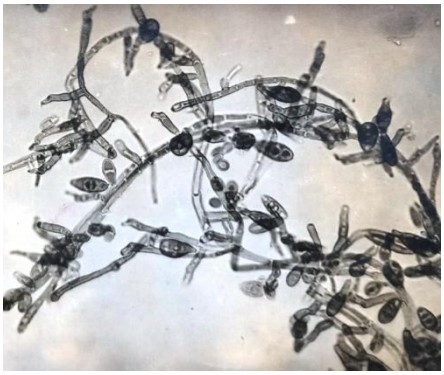
Table 1: Colony diameter (mm) of *A. alternata* in various concentrations of herbicides

Herbicides	Incubation in days	Concentrations (ppm)					
		0	10	100	500	1000	5000
Benthiocarb	1	22.9±1.50	15.1±1.50	14.4±0.50	11.8±0.50	10.4±0.50	8.80±0.25
	3	45.6±1.56	29.7±1.50	27.2±1.00	23.4±1.56	19.8±1.56	17.9±1.56
	5	64.8±2.50	44.4±1.56	39.3±1.56	31.6±1.56	28.7±1.50	25.3±1.56
	7	83.0±1.66	57.5±1.56	50.1±1.66	40.0±1.56	36.7±1.56	33.2±1.56
2,4-D	1	22.9±1.00	22.7±1.50	18.2±1.00	8.0±0.50	0.0±0.00	0.0±0.00
	3	45.6±2.50	42.2±1.50	40.8±1.56	20.5±1.56	11.3±1.56	0.0±0.00
	5	64.8±2.55	68.3±1.66	55.1±1.66	33.3±1.56	21.1±1.56	0.0±0.00
	7	83.0±1.80	89.2±1.66	76.8±1.66	48.2±1.56	25.2±1.56	0.0±0.00
Fluchloralin	1	22.9±1.00	18.4±1.50	17.0±1.56	14.8±1.56	10.7±1.56	0.0±0.00
	3	45.6±1.65	38.7±1.56	32.2±1.50	28.6±1.56	22.0±1.56	0.0±0.00
	5	64.8±1.60	57.2±1.66	46.6±1.50	41.5±1.56	33.3±1.56	7.0±0.50
	7	83.0±2.50	65.8±1.66	59.8±1.66	55.3±1.56	37.8±1.56	19.1±0.50

In addition to affecting the rate of colony growth herbicides also promoted abnormalities in colony structure, sporulation and spore structure (Plate1). At lower concentrations there were no abnormalities in the spore structure. Therefore, for observation of spore abnormalities the highest concentration of treatments in which fungal colony grew was selected. Spores of *A. alternata* growing on untreated PDA had mean length 27.5 um width 9.5 um; number of horizontal septa 5.0 and vertical septa were 3.0 and 2.0 respectively. At 5000 ppm concentration in 2,4-D treated medium sporulation was prohibited and colour of colony became white. Herbicides also changed the spore structure. In 2,4-D treated cultures mean length of spore decreased by 3.27 um and mean width increased by 1.80 um. Number of horizontal septa also decreased but number of vertical septa was not affected. In case of benthiocarb, spore length decreased considerably while no marked change in width was found. In fluchloralin mean length of spore was 17.12 um and mean width 12.65um. In benthiocarb and fluchloralin treated culture the mean number of horizontal septa were 1.0 and no vertical septa was observed.

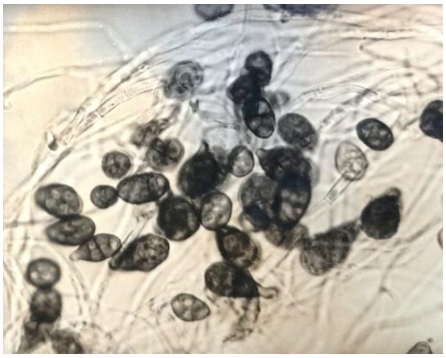


Control



2,4-D


Benthiocarb


Fluchloralin

Germination of spores

Table 2 shows the effect of herbicides on the germination of spore. Under control sets i.e. in the absence of fungicides and herbicides 100% spore germination was recorded after 24 h. All the three herbicides inhibited the spore germination and percentage inhibition increased with increasing concentration of the herbicides in solution. In the case of 2,4-D at the concentration 10, 100, 500 and 1000 ppm the germination at 24 h was 96, 92, 86 and 54 per cent and at 72 h it became 99, 96, 89 and 61 per cent, but at 5000ppm spores did not germinate. Benthiocarb did not have any marked inhibitory effect on spore germination in the different concentrations i.e. 10, 100, 500, 1000 and 5000 ppm after 24 h 92, 92, 90, 89 and 81 per cent spore germinated and after 72 h it came 100, 100, 100, 97 and 90 per cent. In the solution containing 10, 100, 500 1000 and 5000ppm concentration of fluchloralin after 72 h the germination was 99,98,95, 93 and 78 per cent respectively. In the case of herbicides treatment most of the germinable spores in treated sets germinated Within 24 h and a very small proportion germinated afterwards.

Table 2: Percent spores germination of *A. alternata* in various concentrations of herbicides

Herbicides	Incubation in hours	Concentrations (ppm)					
		0	10	100	500	1000	5000
Benthiocarb	24	99.4±1.50	91.6	90.6±1.25	90.0±1.25	88.5±1.25	80.6±1.25
	48	100±1.50	100.0±1.50	99.4±1.25	98.8±1.25	92.0±1.25	80.7±1.25
	72	100±1.50	100.0±1.50	100.0±1.50	100.0±1.50	97.3±1.50	90.0±1.50
2,4-D	24	99.4±1.50	95.8±1.50	91.8±1.50	85.9±1.50	54.2±1.25	0.0±0.00
	48	100±1.50	98.2±1.50	94.1±1.25	88.8±1.50	56.4±1.25	0.0±0.00
	72	100±1.50	99.0±1.50	95.6±1.50	88.9±1.25	61.4±1.25	0.0±0.00
Fluchloralin	24	99.4±1.50	95.8±1.25	93.7±1.25	85.4±1.50	76.0±1.25	70.0±1.25
	48	100±1.50	97.0±1.25	96.1±1.25	91.2±1.25	90.5±1.25	75.9±1.25
	72	100±1.50	98.7±1.25	97.6±1.25	94.8±1.25	93.2±1.25	77.9±1.25

Growth of germ tube

Mean length of germ tube and their elongation rate are given in table 3. Elongation rate of germ tube was generally inhibited by herbicides with exception of 2,4-D and benthiocarb which were stimulatory at lower concentrations. Herbicides had both stimulatory and inhibitory effect on the germ tube growth. Benthiocarb and 2,4-D stimulated the germ tube growth at the concentration of 10 ppm. However, both the herbicides were inhibitory at higher concentrations. Fluchloralin inhibited germ tube growth at all concentrations. At 5000 ppm concentrations of 2,4-D the spores did not germinate, however, at 10, 100, 500 and 1000 ppm the elongation rate of germ tube was 22, 16, 12 and 7 um /h respectively. In case of benthiocarb at the concentrations of 10, 100, 500, 1000 and 5000 ppm the rate of germ tube elongation was 30, 26, 21, 15 and 9 um/h respectively. In fluchloralin treated sets the elongation rate of germ tube in different concentrations i.e. 10, 100, 500, 1000 and 5000ppm was 8, 5, 5, 4 and 1 um respectively.

Table 3: Germ tube growth (µm) of *A. alternata* in various concentrations of herbicides

Herbicides	Incubation in hours	Concentrations (ppm)					
		0	10	100	500	1000	5000
Benthiocarb	24	436.1±2.00	615.2±2.00	419.4±2.00	319.1±2.00	173.8±2.00	82.6±1.00
	48	831.5±3.00	780.5±3.00	725.0±3.00	720.3±3.00	448.8±3.00	208.1±1.00
	72	1675.0±2.00	2182.3±3.00	1878.6±3.00	1529.2±3.00	1114.1±3.00	628.3±3.00
2,4-D	24	436.1±3.00	485.0±2.00	348.3±3.00	251.3±3.00	233.9±3.00	0.0±0.00
	48	831.5±5.00	1310.7±3.00	1071.4±3.00	587.5±3.00	444.4±3.00	0.0±0.00
	72	1675.0±3.00	1695.8±3.00	1151.3±3.00	851.8±3.00	529.7±3.00	0.0±0.00
Fluchloralin	24	436.1±4.00	273.8±2.00	208.6±2.00	204.5±2.00	147.9±2.00	40.3±2.00
	48	831.5±3.00	448.4±3.00	358.3±2.00	272.7±2.00	244.8±2.00	65.8±1.00
	72	1675.0±4.00	578.8±3.00	379.4±3.00	370.6±2.00	285.3±3.00	100.5±2.00

4. Discussion

Herbicides, benthiocarb and fluchloralin inhibited the fungal growth which was dose dependent. However, 2,4-D was stimulatory at low concentration and inhibitory at high concentration. Different herbicides differ in their fungal toxicity has been reported by many workers (Bagga and Kumar, 2000; Altman, 1991; Cohen et al, 1996). Wilkinson and Lucas (1969) reported that herbicides inhibit the linear extension of fungi and form abnormalities in the spore structure. Karr et al., (1979) also noted that herbicides inhibit the growth of *Rhizoctonia solani, Sclerotinia homoeocarpa* and *Drechslera cyanodontis* on agar medium. Both inhibitory and stimulatory effect of 2,4-D on the growth of fungi have been reported by a number of workers Altman and Campbell, 1979; Katan and Eshel, 1973). Hodges (1977) found stimulation of mycelium growth by 2,4-D which was greatest at low concentration. The stimulation in colony growth in 2,4-D treated medium at low concentration may be due to utilization of herbicide as energy source by fungi (chen et al., 1981). A lag phase of 4 days was observed in case of fluchloralin at 5000ppm concentration. It seems the fungus was capable of degrading the herbicides, thus reducing the level of toxicity. The delayed growth phase may also be due to the recovery of the pathogen from deleterious effect implicated by the herbicide. The development of some resistance to the toxic effect of herbicides may also be possible (Abdalia and Manci, 1979).

The germination of spores and germ tube growth are important factors regulating the infection. In all the herbicides treatment percentage spore germination was inhibited which increased with increasing concentration. In 2,4-D and benthiocarb treatments at 10 ppm concentration stimulation in germ tube growth was recorded which is similar to the previous observations of Hodges (1977). 2,4-D is plant hormone and benthiocarb a growth regulator, thus both the chemicals have growth promoting action at low concentration (Caulder et al, 1987). Possibly the stimulation of germ tube of A. alternata is related to these properties of two herbicides.

The results of laboratory study depict at least to a limited extent what may happen in soil or on plants. Stimulation of phytopathogenic fungi at low concentration of 2,4-D noticed in present study and by other workers, prompts to propose that if similar stimulation occurs in the soil where generally the herbicide level are found below 10 ppm the community structure of soil fungi may tend to shift in favour of fungi those stimulated by herbicides. Similar to that it might effect of phytopathogenic fungi on the leaf surface and at low concentration of herbicides diseases severity might increase.

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