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EFFECT OF METAL SALTS ON GROWTH AND ENZYME PRODUCTION OF *ALTERNARIA SOLANI* (ELL AND MART) JONES AND GROUT

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Alternaria solani (Ell & Mart) Jones and Grout is a facultative parasite causing leaf spot and blight diseases of crop plants. Copper sulphate, magnesium carbonate, potassium carbonate and zinc chloride were incorporated in Czpek's dox Agar medium at different concentrations during artificial cultivation of fungus in the laboratory. It revealed that at higher concentrations of these salts, growth and reproduction of *A. solani* were hampered. Relation of enzymes production with growth in metal salts incorporated medium showed less potentiality with regards to both pectinolytic and cellulolytic enzymes adaptibly and constitutively.

INTRDUCTION

Alternaria solani (Ell & Mart) Jones and Grout is a soil brone and air brone facultative parasite. It causes diseases like leaf spot and blight on various crop plants, Various metal salts are used in the medium for artificial cultivation of fungus in the laboratory. There are some changes in growth pattern and enzyme production by the fungus in different metal salts incorporated medium, as reported by Srivastava *et. al* (1959). Trace elements like Iron, Zinc, Manganese, Molybdenum and Calcium are required for growth and sporulation of *Alternaria* sp. as suggested by Madan and Thind (1979). The present investigation was designed to study the effect of metal salts on mycelial growth, sporulation and production of constitutive and adaptive enzymes of pectinolytig and cellulolytic in nature.

MATERIALS AND METHODS

(a) Mycelial growth

(i) Radial growth

The fungus culture was obtained from stock cultures of the laboratory and inoculated in Czapek's Dox Agar medium in 7.5 cm petriplates with 5 mm

diameter inoculum disc of actively growing 7-days old mycelium. The inoculated plates were incubated at 27°C±1°C in a B.O.D. incubator for 7 consecutive days. Two concentrations of each metal salt were taken into account (50 ppm & 100 ppm) and the different salts were sulphate, carbonate and chloride of Copper, Magnesium, and Zinc. These salts were incorporated as 50 ppm and 100 ppm into 25 ml basal medium. These salt incorporated media were poured in 7.5 cm petriplates. Each day after inoculation of the fungus, the radial growth was recorded using milimeter scale and this was continued upto 7 days. In each cage, fungus was grown in normal medium as conttol and 3 replications were kept in each case.

Dry weight of mycelium

For this purpose, the fungus was grown in 50 ml Czapek's Dox broth taken in 250 ml conical flasks, inoculated with 5 mm diameter inoculum disc of actively growing 7 days old mycelium and incubated at 27°C±18 for 7 consecutive days in a B.O.D. incubator. After 7th day of inoculation, the content of the flask was strained in glass funnel providing with a four fold filter paper. The fungal mat was then blot dried thrice, After blot drying, the material was taken in a previously weighed filter paper and subjected to oven dry at 60°C for 24 hours. After substracting the weight of the filter paper the actual weight of the myeelial mat was obtained. Parallel controls (without addition of test chemicals) were also done. In each case three replications were taken.

Sporulation

To study the effect of different metal salts on the sporulation of *Alternaria solani*, the fungus was grown in 7 5 cm petriplants containing 25 ml of Cxapek's Dox agar medium alone (control) and wlth 50 and 100 ppm of the salts. The petriplates were inoculated as stated previously and incubated at 27°C±1°C for 7 days.

On 7th day of incubation, 5 ml of sterile distilled water was added on each petri-plates. These plates were stirred with a sterile needle and the supernatant was collected in a separate tube and the concentration of spore per millilitre was counted using Haemacytometer. In this case, three replicat ons were taken.

Enzyme activity

To study the effect of 4 chemicals on production of adaptive pectinolytlc enzyme by *Alternaria solani*, the fungus was grown in Cxapek's Dox broth containing Copper sulphate, Magnesium carbonate, Potassium carbonate and Zinc chloride at 50 ppm and 100 ppm. For adaptive nature of pectinolytic enzyme production, the medium was enriched with 1.2 percent pectin (apple pectin) and the flasks were inoculated as stated previously. The inoculated flasks were incubated at

26°±1°C. for 7 days. After incubation the mycelial met was harvested and the culture filtrate thus obtained were directly centrifuged at 5000 rpm at 5°C for 20 minutes. The supernatants were thus obtained used as crude enzyme preparations. Pectinolytic enzymes were assayed by 1.2 percent pectin (apple pectin) buffered with 0.1 M citrate buffer in reaction mixture. Assay was made viscosimetrically with the help of a 10 ml Ostwald viscosimeter. Data were recorded by the loss of viscosity in percentage basis by the enzyme preparation at a given time with the following formula.

$$\% \text{ Loss in viscosity} = \frac{No - Nt}{No - Nw} \times 100$$

where, No-viscosity at initial stage, Nt-viscosity at given time, Nw—viscosity of water.

For cellulase assay, all the procedures were the same excepting the pectin was substituted by 1.2 percent carboxy methyl cellulose and this was incorporated in the basal medium. Both adaptive and constitutive enzymes were essayed viscosimetrically. In all the cases control experiments (without test chemicals and no pectin or carboxy methyl cellulose) were run side by side.

RESULTS AND DISCUSSIONS

Data are presented in Table 1 for radial growth in mm, mycelial weight in mg in Table 2, sporulation in Table 3, adaptive pectinolytic and cellulase in Table 4 and constitutive pectinolytic and cellulolytic enzymes in Table 5 respectively.

Table 1. *Radial growth of* Alternaria solani *(in mm) recorded at twenty four hours interval. Data are average of 3 replications.*

Test chemicals	Cons. used (in ppm)	Days after inoculation Radial growth (in mm) 1	2	3	4	5	6	7
Copper sulphate	50	—	13.7	14.5	17.7	22.4	23.5	26.2
	100	—	12.3	14.6	18.5	20.0	22.0	22.4
Magnesium carbonate	50	—	14.9	21.6	28.4	34.8	35.5	36.2
	100	—	12.5	14.0	17.1	20.4	24.5	27.0
Potassium carbonate	50	—	13.4	17.9	24.6	29.6	30.6	32.0
	100	—	11.5	15.9	20.5	2o.6	27.3	29.8
Zinc chloride	50	—	12.2	15.6	18.9	22.1	24.6	28.9
	100	—	10.2	13.8	17.0	21.5	23.0	25.0
Control			19.0	27.0	33 5	39.0	44.5	47.6

CD (0.05 p)=0.023

From the above Tables 1-3 it was observed that all the four chemicals reduced the radial growth, sporulation and dry mycelial weight of *Alternaria solani*. From

the above study it might be concluded that the fungus did not tolerate these chemicals with regards to its growth and sporulation.

Chemicals like Copper sulphate, Magnesium carbonate, Potassium carbonate and Zinc chloride in higher concentration might restrict the growth of the fungus due to toxicity. This toxicity may lead to disruption in metabolic pathways, enzyme production and phenolic substances which may causes the autolysis of the fungus. The observations were in agreement of the previous reports by Capellini (1966), Carlton (1953) and Macan and Jhilld (1979).

Table 2. *Effect of selected chemicals on dry mycelial weight (in mg) of* Alternaria solani. *Data are the average of three replication .*

Chemicals	Conc. (ppm)	
	50	100
Copper sulphate	46.0	43.3
Magnesium carbonate	72.3	38 6
Potassium carbonate	44 3	30.3
Zinc chloride	77.0	33.6
Control (No chemicals)	85.0	—

CD (0.05)=58.8

Table 3. *Effect of selected chemicals on sporulation of* Alternaria solani *(data are average of three replication).*

Test chemicals	Sporulation (x 10^6) on the 7th day growth at conc. (ppm) per ml of spore suspension	
	50	100
Copper sulphate	1.5	1.3
Magnesium carbonate	1.1	0.3
Potassium carbonate	5.0	0.7
Zinc chloride	1.85	0 62
Control	3.75	—

CD (0 05 P) = 0.011

From the Table 4 it was observed that there was a general tendency to produce pectinolytic enzymes which were adaptive in nature. In control flasks, the production of adaptive pectinolytic enzyme was more in comparison to the flasks, incorporated with Copper sulphate, Magnesium carbonate, Potassium carbonate and Zinc chloride at 50 and 100 ppm concentrations of which the flasks containing Copper sulphate, there was lesser production of adaptive pectinolyt;c enzyme than any other chemicals incorporating flasks by the fungus. In higher concentrations, the production of the enzymes was too less due to inactivation of enzyme producing system of *Alternaria solani*.

From the same Table (Table 4) it was clear that *Alternaria solani* can produce cellulase adaptively when the medium was enriched with 1.2 percent carboxy methyl cellulose. But when the concentration of different salts was increased in the medium, the ability of producing enzymes decreased. This might be due to toxic nature of the salts which inhibited or disrupted the enzyme producing system of the fungus. Similar data were reported by Honck and Miller (1965) and Mehta (1977).

Table 4. *Loss in viscosity of adaptive enzymes (pectinolytic and celluloytic) secreted by* Alternaria solani *in response to selected chemicals (Figures are the average of three replications).*

Test Chemicals	Conc. (ppm)	% Reduction in viscosity after minutes									
		15		30		45		60		90	
		a	b	a	b	a	b	a	b	a	b
Copper sulphate	50	2.6	5.0	10.5	12.0	15.0	23.7	13 1	30.5	13.1	30 5
	100	4.0	3.3	5.5	12.0	8.0	16 9	8.0	23.7	8.0	26.5
Magnesium carbonate	50	7.0	35.2	8.9	39.1	10.6	49.0	15.9	54.9	21.0	54 9
	100	4.6	12.3	8.5	16.3	12.3	22.4	16.0	26.5	18.0	34.0
Potassium carbonate	50	2.3	19.8	9.5	29.4	14.2	39.2	16.0	44.7	19.0	47.0
	100	4.5	18.5	9.5	25.0	11.3	27.2	15.0	31.2	15.0	37.5
Zinc chloride	50	2.4	17.9	8.5	26.0	14.0	31.2	18.2	35.4	19.5	38 5
	100	4.0	14.8	6.5	18.8	9.5	26.0	11.2	29.7	11.2	34.6
Control	(a)	32.1		47.3		62.9		75.0		75 0	
	(b)	26.0		32.1		47.3		62.9		70.0	

(a) Pectinolytic enzymes, (b) Cellulolytic enzymes.

Table 5. *Loss in viscosity of constitutive enzymes (pectinolytic and cellulolytic) secreted by* Alternaria solani *in response to selected chemicals (Data are the average of three replications)*

Test Chemicals	Conc. (ppm)	% Reduction in viscosity after minutes									
		15		30		45		60		90	
		a	b	a	b	a	b	a	b	a	b
Copper sulphate	50	6.6	15.9	13.2	20.3	15.0	23.8	17.3	25.6	17.3	28.3
	100	2.0	11.0	12.4	17.2	13.2	20.3	13.9	21.5	15.3	23.1
Magnesium carbonate	50	5.8	22.3	12.4	25.2	14.5	27.1	16.0	32.0	17.0	36.8
	100	1.0	16.5	3.0	19.0	5.3	22.4	9.2	26.5	9.8	31.0
Potassium carbonate	50	4.9	22.3	6.6	28.1	7.1	34.9	7.4	36.8	8.3	38.8
	100	2.2	9.9	3.5	11.5	4.3	14.1	4.3	15.9	4.8	23.8
Zinc chloride	50	5.8	11.5	9.2	24.8	12.9	29.2	14.8	33.6	15 0	33.6
	100	3.7	7.7	6.3	10.6	7.5	22.2	8.9	29.1	9.0	32.0
Control	(a)	28.0		39.5		46.5		55.0		58.0	
	(b)	28.3		41.5		51.3		54.0		60.1	

(a) Pectinolytic enzyme, (b) Cellulolytic enzyme.

From Table 5, it may be pointed out that the fungus had less ability to produce pectinolytic enzymes of its own. Though in control flasks, there was a tendency to produce constitutive pectinolytic enzymes by the fungus in a lesser quantity in compansion to adaptive nature of pectinolytic enzyme production. Further more, it might be noted that production of constitutive cellulase by the fungus was more than that of pectinolytic enzyme in both the concentrations of salts (50 ppm and 100 ppm). Lodha (1976) and Srivastava (*et al* 1959) also pointed out the similar observations.

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REFERENCES

Capellini, R. A. (1966). Growth and polygalacturonase production by *Rhizopus stoloni fer*. *Phytopath*. 56, 734-737.

Charlton, K. M. (1953). The sporulation of *Alternaria solani* in culture. *Trans. Br. Mycol.* Sec. 36, 348-355.

Hancok, J. G. and Miller, R. L. (1965). Association of cellulolytic, pectolytic and Xylolytic enzymes with southern anthracnose, spring black stem and *Stemphylinm* leaf spot of alfa alfa, *Phytopath*. 55, 356-360.

Lodha, P. C. (1976). Note on the chemical control of early blight of potato. *Ind. J. Agric. Sci.* 46, 605-606.

Nadan, M. and Thind, K. S. (1979). Role of trace elements on the growth and spcrulation of *Alternaria charatanum* and *Alternaria solani*. *Proc. Ind. Acad. Sci.* 45, 628-632.

Mehta, P. (1977). Fungicides inhibitory agents of polygalacturonase. *Ind. Phytopath.* 30, 539-541.

Srivastava, D. N., Echandi, E. and Walker, J. C. (1959). Pectolylic and cellulolytic emzymes produced by *Rhizopus stolonifer*. *Phytopath*, 49, 145-148.

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