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Adaptation of *Botryodiplodia theobromae* (Pat) Griff, ET.maubl to Salts and Its Importance in Agriculture*

by

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Introduction: The use of certain drugs particularly antibiotics against bacteria and certain insecticides against insects have resulted in the appearance of resistant strains, creating a new problem in the treatment of diseases. Unlike insects so far fungi do not seem to have reacted to fungicides in such a spectacular manner although a downy mildew resistance does occur against certain chemicals (Taylor 1953). Fungi have been reported to educate themselves to increasing concentrations of toxic substances (Wilson 1947 and Hirschhorn and Munneck 1950). In some cases mutation clearly accounted for what could have been considered adaptation while in other cases visible mutation did not account for such results. The present work was undertaken to study the adaptation of *Botryodiplodia theobromae* to copper sulphate, mercuric chloride and zinc sulphate.

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Materials and Methods: The fungus *B. theobromae* (causing fruit rot of papaya) was taken in the present study. Pathogenicity tests were carried out and pure culture was established in papaya dextrose agar medium. The concentrations of the salts used in the present investigation were: Copper sulphate 0, 100, 200, 300, 500, 700, 1000 ppm and 1200 ppm, mercuric chloride, 0, 10, 20, 30, 50, 70, 100 and 120 ppm and zinc chloride 0, 100, 200, 300, 500, 700, 1000 and 1200 ppm in the form of copper, mercury and zinc as active ingredients. The pH of the medium was maintained at 5 throughout the experiment. The inoculated petriplates were incubated at 25°C.

Results: Diameter of the colonies of *B. theobromae* after 48 hours on media containing increasing concentrations of copper sulphate is furnished in Table 1.

TABLE 1. *Growth of B. theobromae on media with different concentrations of copper*

Sources of inoculum	Average diameter of colony in mm							
	Concentrations sulphate of copper in ppm							
	0	100	200	300	500	700	1000	1200
Papaya dextrose agar (PDA)	88	69	55	45	31	—	—	—
100 ppm medium of the first series	90	72	61	48	33	4	—	—
200 ppm medium of the second series	92	74	65	52	37	13	6	—
300 ppm medium of the third series	92	77	69	56	42	18	12	—

In copper sulphate, colony size gradually decreased on PDA from 100 to 500 ppm in comparison to control *i.e.* 0 ppm. At concentration of 700 and above, there was no fungus growth indicating the toxicity of copper sulphate to the fungus.

Inoculum from 100 ppm of copper sulphate in first series, 200 ppm copper sulphate in second series and 300 ppm copper sulphate of third series were separately transferred to PDA containing 0, 100, 200, 300, 500, 700, 1000 and 1200 ppm of copper sulphate to constitute the 2nd, 3rd and 4th series of experiments respectively under Table 1.

It is interesting to note that colony size gradually decreased on PDA containing 500 ppm and above indicating clearly the toxicity of copper sulphate to the fungus. Having grown on 100 ppm for one generation the fungus grew comparatively to a larger size of colony upto 500 ppm in 2nd series and unlike first series grew even in 700 ppm in 2nd series. Likewise, after growing for two generations in copper sulphate the fungus grew better in 3rd series in comparison to 2nd series exhibiting nearly three fold growth under 700 ppm and also grew in 1,000 ppm eventually on account of its acquiring adaptation. This is further confirmed in the 4th series wherein

after being grown for three succesive generations in copper sulphate the fungus grew better in all concentrations upto 1000 ppm except 0 ppm.

It would be seen from the table that adapted line showed better growth than the unadapted line when both were transferred to medium of copper sulphate.

The growth measurements of *B. theobromae* after 48 hours on media containing increasing concentrations of mercuric chloride is given in Table 2.

TABLE 2. *Growth of B. theobromae media containing different concentrations of mercury.*

Source of inoculum	Average diameter of colony in mm							
	Concentrations of mercuric chloride in ppm							
	0	10	20	30	50	70	100	120
Papaya dextrose agar	87	61	54	41	19	7	—	—
10 ppm medium of the first series	90	64	57	48	23	14	—	—
50 ppm medium of the second series	91	67	59	52	27	21	5	—
70 ppm medium of the third series	93	70	62	57	37	25	10	—

Colony size gradually decreased on PDA from 10 to 70 ppm of mercuric chloride in comparison to control *i. e.* 0 ppm. At concentration of 100 ppm and above fungus growth did not occur indicating clearly the toxicity of mercuric chloride to the fungus. Inoculum from 10 ppm of mercuric chloride of 1st series, 50 ppm of mercuric chloride of 2nd series and 70 ppm of mercuric chloride of 3rd series were separately transferred to PDA containing 0, 10, 20, 30, 50, 70, 100 and 120 ppm of mercuric chloride to constitute 2nd, 3rd and 4th series of experiments respectively under Table 2.

It is interesting to note that colony size gradually decreased on PDA from 10 to 70 ppm of mercuric chloride with no growth on PDA containing 100 ppm mercuric chloride and above indicating clearly the toxicity of mercuric chloride to the fungus. Having grown on 10 ppm for one generation, the fungus grew comparatively to larger size of colony upto 70 ppm in 2nd series. Likewise after growing for two generations in mercuric chloride the fungus grew better in 3rd series in comparison to 2nd series exhibiting nearly three fold growth in 70 ppm and growth in 100 ppm of mercuric chloride evidently on account of its acquiring adaptation. This is further confirmed in the 4th series wherein after being grown for three successive generations in mercuric chloride the fungus grew better in all concentrations upto 100 ppm except under 0 ppm.

It, therefore, appeared that unadapted line of fungus could only tolerate a concentration of 70 ppm of mercurie chloride in PDA but the adapted line could tolerate a concentration of 100 ppm of mercuric chloride in PDA.

The growth measurement of *B. theobromae* after 48 hours on medium containing increasing concentrations of zinc sulphate is furnished in Table 3.

TABLE 3. *Growth of B. theobromae on media containing different concentrations of zinc*

Source of inoculum	Average diameter of colony in mm							
	Concentration of zinc sulphate in ppm							
	0	100	200	300	500	700	1000	1200
Papaya dextrose agar	89	81	73	45	33	16	—	—
100 ppm medium of the first series	92	83	79	53	37	25	8	—
300 ppm medium of the second series	86	84	80	60	41	29	13	8
700 ppm medium of the third series	94	85	88	64	44	34	17	12

Colony size gradually decreased on PDA from 100 to 700 ppm of zinc sulphate. At concentration of 1000 and above no growth occured indicating clearly the toxicity of zinc sulphate to the fungus. Inoculum from 100 ppm of zinc sulphate of first series, 300 ppm of zinc sulphate of 2nd series and 700 ppm of zinc sulphate of 3rd were separately transferred to PDA containing 0, 100, 200, 300, 500, 1000 and 1200 ppm of zinc sulphate to constitute the 2nd, 3rd and 4th series of experiment under Table 3.

It is interesting to note that the colony size gradually decreased on PDA from 100 to 700 ppm of zinc sulphate with no growth on PDA containing 1000 ppm of zinc sulphate and indicating clearly the toxicity of zinc sulphate to the fungus. Having grown on 100 ppm for one generation the fungus grew comparitively to larger size of colony upto 700 ppm in second series and unlike first series grew even in 1000 ppm in 2nd series. Likewise after growing for two generations in zinc sulphate the fungus grew better in 3rd series in comparison to 2nd exhibiting good growth in 1000 ppm and 1200 ppm evidently on account of its acquiring increased adaptation. This is further confirmed in the 4th series wherein after being grown for 3 successive generations in zinc sulphate, the fungus grew better in all concentrations upto 1200 ppm except under 0 ppm.

It, therefore, appears that unadapted line of fungus could only tolerate a concentration of 700 ppm zinc sulphate whereas adapted line tolerated 1200 ppm of zinc sulphate.

Behaviour of unadapted and adapted lines on mercuric chloride and zinc sulphate when grown on the PDA containing increasng concentrations of copper sulphate is shown in Table 4.

TABLE 4

Source of inoculum	Average diameter of colony in mm							
	Concentrations of copper sulphate in ppm							
	0	100	200	300	500	700	1000	1200
Papaya dextrose agar	92	67	55	46	31	—	—	—
50 ppm of medium of the third series from the Table 2 (Mercuric chloride)	89	67	58	43	36	—	—	—
700 ppm medium of the third series Table 3 (Zinc sulphate)	88	63	56	42	37	—	—	—

Behaviour of the unadapted and adapted lines on copper sulphate and zinc sulphate of fungus when grown on PDA containing concentration of mercuric chloride is furnished in Table 5.

TABLE 5

Source of inoculum	Average diameter of colony in mm							
	Concentration of mercuric chloride in ppm							
	0	10	20	30	50	70	100	120
Papaya dextrose agar	87	81	54	39	23	9	—	—
300 ppm medium of the third series from Table 1 (Copper sulphate)	89	68	53	42	20	13		
700 ppm medium of the third series from Table 3 (Zinc sulphate)	93	71	52	47	25	11	—	—

Behaviour of unadapted and adapted line of copper sulphate and mercuric chloride when grown on PDA containing increasing concentrations of zinc sulphate is given in Table 6.

TABLE 6

Source of inoculum	Average diameter of colony in mm							
	Concentration of zinc sulphate in ppm							
	0	100	200	300	500	700	1000	1200
Papaya dextrose agar	91	82	75	49	30	13	—	—
300 ppm medium of the third series Table 1 (Copper sulphate)	95	84	70	53	37	11	—	—
70 ppm medium of the third series Table 2 (Mercuric chloride)	88	81.5	73	54	36	12.5	—	—

In these experiments, the unadapted line of fungus from PDA and line adapted on the mercuric chloride and zinc sulphate series were transferred to medium containing increasing concentrations of copper sulphate (Table 4). The line adapted on copper sulphate and zinc sulphate of 3rd series were transferred to medium containing increasing concentrations of mercuric chloride (Table 5) and the line adapted on copper sulphate and mercuric chloride of 3rd series were transferred to medium containing increasing concentrations of zinc sulphate (Table 6). The inoculum used for adapted lines from the medium containing 700 ppm of zinc sulphate, 50 ppm of mercuric chloride and 300 ppm of copper sulphate in PDA were transferred respectively to copper sulphate, zinc sulphate and mercuric chloride series. (The source is the same as in Table 1, 2 and 3).

Tables 4, 5 and 6 reveal that the lines which had earlier distinctly adapted to mercuric chloride, zinc sulphate and copper sulphate now behaved like the unadapted line directly transferred from PDA to copper sulphate, mercuric chloride and zinc sulphate series. This means the line adapted in respect of one salt only having adapted on it showed no signs of adaptation for other salts.

Discussion: In the present study the fungus developed capacity for adaptation when grown in media of ascending toxicity. This capacity of "drugfastness" further developed in descending generation. Similar results have been reported by various workers like Hirschhorn and Munneck (1950), Wilson (1947), Parry and Wood (1958 and 1959), Littauer and Gutter (1953) and Gattani (1951). Gattani further maintains that it is essential that fungicides should be alternated so that formation of tolerant strains does not take place or is eliminated. Jurkowaska (1952) working on *Aspergillus niger* found that when this fungus became tolerant to copper sulphate it automatically developed tolerance to zinc and nickel. The present work revealed results contrary to the findings of above worker. It was found that fungus line tolerant to copper sulphate was vulnerable to mercuric chloride and zinc sulphate in their various concentrations. This is in confirmation with the work of Ramel Kamp and Maxon (1942) on *Steplylococcus aurens*, where they found that the response of an organism to therapeutic action of sulphonamide drugs is not affected on account of the development of their resistance to penicillium.

Finally it is concluded that it is essential to alternate the fungicides and prevent drugfastness as otherwise constant exposure to one type of fungicides may give rise to drugfast strains on which any amount of toxicity of that particular fungicide will have no detrimental effect.

Summary: Studies on adaptation of *B. theobromae* to copper sulphate, mercuric chloride, and zinc sulphate indicated that (1) fungus developed resistance to the increasing concentrations of the salts of various metals used when it was gradually transferred from media containing lower concentrations to higher concentrations of the toxic salts and (2) fungus culture that got adapted to mercuric chloride did not retain the property of adaptation to copper and zinc sulphates indicating that adaptation to one chemical does not necessarily mean adaptation to other chemicals.

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