ON THE INFLUENCE OF SULPHA DRUGS IN SUGARCANE

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I. Introduction

The utility of sulpha drugs as bacteriastatic agents in animal physiology is well known. Many workers from time to time have tested the effect of these drugs on different plants and plant processes as well. However, the results obtained were quite diverse and variable. Thus, Sulphonamides were found to inhibit germination of oats and wheat (Bustinza and Ahvaro, 1945; Bhardwaj & Rao, 1954) as also of other higher plants (Euler, 1948). On the other hand, improved germination was found in *Hibiscus esculentus* (Kumar & Srivastava, 1953). Increased seedling mortality has also been reported by Bhardwaj and Rao (1954). The inhibiting action of Sulphonamides on root growth has been observed by a host of workers covering a wide range of higher plants (Grace, 1938; Woods, 1940; Macht and Kehoe, 1943; Euler, 1948; Andus and Quastel, 1948; Kayser and Benson, 1948; Bhardwaj and Rao 1954; Bhardwaj, 1954). Stimulating effect on root growth has also been recorded in *Hibiscus esculentus* (Kumar and Srivastava, 1953) and sugarcane (Rao, Prasad and Khanna, 1956). Both inhibition (Andus and Quastel, 1948) and stimulation (Kumar and Srivastava, 1953) were regarded as distinct from Sulphonamide bacteriastasis. Inhibited growth of seedlings has been observed in barley, rye, *Lolium perenne*, *Phleum pratense*, *Lepidium sativum*, *Vicia faba* (Euler, 1948), flax (Moore, 1953) and wheat (Bhardwaj and Rao, 1954). On the other hand, Rao, Prasad and Khanna (1956) have reported stimulated shoot growth in sugarcane. Bhardwaj and Rao (1954) observed that tillering was induced early and earing delayed by 30 days with shrivelling of grains in wheat. Rao *et al.* (1956), working on sugarcane, found general improvement in stalk weight, leaf number, fresh weight of roots, stem and whole plant, dry weights of sheath and root, moisture content of leaf, sheath and whole plant as also in yield and juice quality resulting from sulphanilamide treatment. Garo (1947) showed in *Hyapolum senatum*, depression of transpiration due to complete closing down of the stomata when treated with Sulphamides. Spraying of wheat seedlings with the drug solution led to chlorosis and even necrosis; however, the plants recovered in about 7 days (Hotson, 1953). Woods (1950) stated that the primary action

of Sulphamides is competitive inhibition of an enzyme reaction (or reactions) in which PABA is involved; the chief function of PABA is its requirement in the synthesis of folic acid and the inhibition of this reaction is the principle point of attack of the drugs in the cell. Moore (1953) interpreted this results as suggesting that in the flax seedlings Sulphanilamide interferes with the conversion of pamino benzoic acid to Pteroyl-glutamic acid. Sivaramakrishnan and Sharma (1953) have also studied the effect of sulpha drugs on the pamino benzoic acid metabolism with lebelled C^{14} in *Phaseolus radiatus*. Audus and Quastel (1948) thought that the effect in general varied with the drug and its concentration.

The above results showed the varied responses obtained in different crops, leading to the conclusion that the influence of these sulpha drugs varied largely with species and as such it was thought relevant to analyse its influence on the growth of sugarcane.

II. MATERIALS AND METHODS

One budded sets of variety B.O. 17 were planted in February in pots filled with the local calcareous loamy soil. A basal dose of nitrogen (as ammonium sulphate) and Phosphorus (as single super) at 60 and 75 lb./acre respectively was applied at the time of planting. Five sulpha drugs, *viz.*, Sulphanilamide, Sulphadiazine, Sulphaguanidine, Sulphamerazine and Sulphathiazol were under study. Two doses (184 and 368 p.p.m.) of each drug were applied at the third month stage to the soil; another dose of 122 p.p.m. was applied at the 4th month stage to another set of pots. In all 10 cultures for treatment were maintained. Irrigations and hoeings whenever necessary were given to maintain optimum conditions of growth.

Periodical observations on growth and other attributes were recorded along with yield and juice quality at harvest.

III. EXPERIMENTAL FINDINGS

Within a week of application of the drugs, leaves started yellowing—degree of chlorosis being directly proportional to the dose, with specific variation among the different drugs. Thus in Sulphanilamide, Sulphaguanidine and Sulphadiazine, all the leaves became entirely chlorotic while in Sulphathiazol it was confined only to the intercostal regions (Fig. 1). In Sulphamerazine there was just a suggestion of chlorosis. At the lowest dose of 122 p.p.m., chlorosis appeared only with Sulphanilamide. But by the end of the 3rd month, leaf colouration was restored.

All the drugs in all concentrations tried retarded root growth, the retardation being directly proportional to the concentration of the drug (Fig. 2).

Shoot growth in turn was also retarded and the effect varied with the drug and its concentration. In case of Sulphanilamide (184 and 368 p.p.m.), Sulphaguanidine (368 p.p.m.) and Sulphadiazine (368 p.p.m.) plants stopped growing altogether and died off within a month, least inhibition being under Sulphamerazine (184 p.p.m.) and Sulphathiazol (184 p.p.m.). With 122 p.p.m. dose, growth was retarded only with Sulphanilamide; with others it was as good as that of control. With the passage of time, the treated plants picked up the rate of growth and by the end of the 5th month, cultures under Sulphamerazine (184 p.p.m.) were on par with control (Table I).

TABLE I

Height of mother shoot in inches

Drugs	Dose	Age in days				
		90	113	140	173	205
Sulphaguanidine ..	Control	5·47	13·80	19·81	30·60	40·40
	1·5 gm.	5·50	8·40	16·11	27·11	36·40
	3·0 gm.	5·50	6·50	..	..	..
Sulphadiazine ..	Control	6·60	16·34	21·5	33·24	40·2
	1·5 gm.	5·87	8·7	12·67	25·17	32·97
	3·0 gm.	5·47	6·50	..	..	..
Sulphathiazol ..	Control	6·8	12·0	19·5	29·17	36·2
	1·5 gm.	7·6	13·4	18·0	23·4	30·57
	3·0 gm.	7·77	12·57	17·24	19·0	25·4
Sulphamerazine ..	Control	9·51	18·50	26·50	36·80	45·5
	1·5 gm.	8·91	14·40	25·50	37·8	45·67
	3·0 gm.	7·60	8·50	15·20	25·2	32·67

Within a fortnight after the application of the drug, the tissues at the lower nodes in all the treatments, excepting Sulphamerazine (both doses) and Sulphathiazol (184 p.p.m.), became meristematic and produced an undifferentiated mass of tissues, with a few distinct bud forms. Many of these germinated later on, to form healthy tillers (Fig. 3). The quantity of adventitious tissue formed, again varied with the drug and its concentration. In decreasing order, the drugs can be arranged as follows: Sulphanilamide, Sulphadiazine and Sulphaguanidine, Sulphathiazol and Sulphamerazine. With the lower dose of 122 p.p.m., no adventitious tissue was formed except with Sulphanilamide where, in a few plants, mild symptoms of such

structures were visible. However, in all the plants treated with Sulphanilamide, bud size and enlarged and many of them had sprouted along with the adjoining root band to form water-suckers. The number of green leaves also varied with the drug and was inversely proportional to its concentration (Table II).

TABLE II

Number of green leaves (Mother shoot)

Drugs	Dose	Age in days				
		90	113	140	173	205
Sulphaguanidine ..	Control	0·67	0·67	1·34	1·34	2·67
	1·5 gm.	1·00	1·00	1·67	1·00	2·00
	3·0 gm.	1·00	1·00	..	..	..
Sulphadiazine ..	Control	0·34	0·34	2·00	1·00	1·34
	1·5 gm.	0·67	0·67	1·00	1·34	1·34
	3·0 gm.	0·67	0·67	..	..	..
Sulphathiazol ..	Control	0·34	0·34	0·34	0·34	0·34
	1·5 gm.	1·00	1·00	1·00	1·00	1·00
	3·0 gm.	0·67	0·67	0·67	1·00	1·00
Sulphamerazine ..	Control	0·67	0·67	0·67	0·67	0·67
	1·5 gm.	1·00	1·00	1·00	0·07	1·00
	3·0 gm.	0·67	0·67	0·67	0·34	0·34

The drugs showed a general tendency to improve the number of healthy tillers per clump (Table III)—the best result having been obtained with Sulphamerazine and Sulphathiazol (184 p.p.m.). However, an indication of higher doses being toxic was clearly discernible. Here the cluster of tillers formed by the sprouting of buds in the adventitious mass as excluded, most of which ultimately died off, due probably to competition among themselves.

The weight of canes per clump decreased steadily with increase in concentration of the drug—the only treatment having equalled the value of control being 184 p.p.m. dose of Sulphamerazine (Table IV).

So far as the various quality attributes like extraction %, Brix, sucrose, purity and glucose were concerned, no systematic trends were discernible (Table V).

TABLE III

Total number of tillers per clump

Drugs	Treatments	Age in days				
		90	113	140	173	205
Sulphaguanidine ..	Control	0·67	0·67	1·34	1·34	2·67
	1·5 gm.	1·00	1·00	1·67	1·00	2·00
	3·0 gm.	1·00	1·00	..	..	..
Sulphadiazine ..	Control	0·34	0·34	2·00	1·00	1·34
	1·5 gm.	0·67	0·67	1·00	1·34	1·34
	3·0 gm.	0·67	0·67	..	..	..
Sulphathiazol ..	Control	0·34	0·34	0·34	0·34	0·34
	1·5 gm.	1·00	1·00	1·00	1·00	1·00
	3·0 gm.	0·67	0·67	0·67	0·67	0·67
Sulphamerazine ..	Control	0·67	0·67	0·67	0·67	0·67
	1·5 gm.	1·00	1·00	1·00	0·67	1·00
	3·0 gm.	0·67	0·67	0·67	0·34	0·34

TABLE IV

Yield in Oz./Clump

Sl. No.	Drugs	Control	Dose		
			1·0 gm.	1·5 gm.	3·0 gm.
1	Sulphanilamide	9·7	4·3	..	..
2	Sulphadiazine	7·7	6·7	6·5	3·0
3	Sulphaguanidine ..	9·3	8·3	5·7	4·7
4	Sulphamerazine ..	7·3	6·7	7·3	5·0
5	Sulphathiazol	7·3	6·7	4·0	3·3

IV. DISCUSSION

Application of various sulpha drugs to cane brought about interesting effects. The first symptom to appear was chlorosis. This may be possibly

TABLE V

Juice characteristics

Drugs	Dose	Extraction %	Glucose	Brix	Sucrose	Purity
Sulphaguanidine	Contro.	55·12	0·888	20·6	14·317	68·08
	1·0 gm.	50·00	0·783	18·8	15·536	78·08
	1·5 gm.	..	0·816	20·7	15·558	75·83
	3·0 gm.	..	2·203	22·5	14·220	63·78
Sulphanilamide	Control	55·12	0·725	19·6	15·631	79·85
	1·0 gm.	50·00	0·788	20·2	16·150	81·16
Sulphadiazine..	Control	56·71	1·672	20·0	16·557	82·87
	1·0 gm.	59·97	0·659	19·6	15·822	78·27
	1·5 gm.	62·50	0·636	21·75	16·499	76·71
	3·0 gm.	50·00	1·813	19·5	14·979	76·82
Sulphamerazine	Control	53·40	0·152	23·3	19·03	78·95
	1·0 gm.	50·00	0·202	20·5	16·130	78·45
	1·5 gm.	50·00	0·269	21·9	15·223	71·29
	3·0 gm.	50·00	..	21·2	13·962	66·26
Sulphathiazol..	Control	52·94	..	21·3	17·271	83·84
	1·0 gm.	50·00	..	21·3	15·227	72·38
	1·5 gm.	66·75	..	21·0	18·137	64·31
	3·0 gm.	51·38	..	21·0	14·775	70·62

due to a retarded absorption of essential elements, like magnesium and iron for the formation of chlorophyll, resulting in chlorosis and consequent inhibited carbon assimilation, ultimately leading to retarded root and shoot growth. Since the normal metabolic state of leaf is disturbed, a retarded production of auxins may also be visualised. Snow (1932) in *Picum sativum* found that the larger leaves have also an inhibitory effect on lateral buds and its power increases with the age of the seedling. Thimann and Skoog (1934 *a*, 1934 *b*) observed that in the leaves also growth substances are produced but in lesser amounts than in the bud. Looking at the extent of foliage developed in sugarcane, if it is presumed that production of growth substances is considerable, the retardation in the production rate of these auxins might explain to a certain extent the sprouting of lateral buds and the production of adventitious tissues on the nodes. However, the explanation does not seem to be plausible, since retarded root and shoot growth, though reported by many workers as stated earlier, has not been accompanied with chlorosis

except in wheat (Hotson, 1953) and even there when the plants were sprayed aerially.

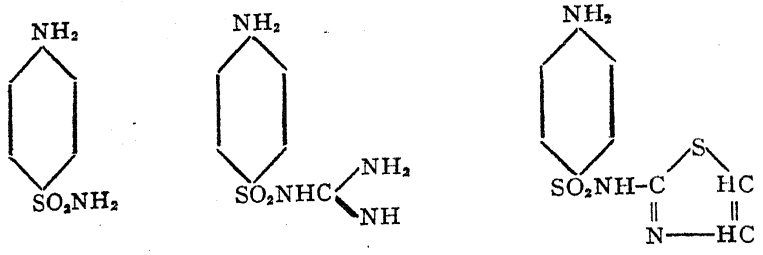
Alternatively, a disturbance in the enzymatic complex may also be put forward. These sulpha drugs are known to form metal complexes and if they do so with the prosthetic groups of enzymes it might result in chlorosis and might also explain other phenomena observed, *viz.*, retarded root and shoot growth, sprouting of lateral buds and production of adventitious tissues on the nodes, in as much as these changes are the basis of the physiological effects caused by auxins (Overbeek, 1952). The observations recorded thus seem clearly to bring auxins into the picture. However, Audus and Quastel (1948) thought that root growth inhibition caused by these drugs is a phenomenon quite distinct from that produced by plant growth substances. It seems that synthesis of auxins in the apical bud and leaves is either retarded or is rendered inactive as soon as it is produced, resulting in the reduction of apical dominance and leading to sprouting of lateral buds and production of an uncontrolled mass of adventitious tissues. Went (1951) observed that deficient auxin supply in the first place prevents normal elongation of the main shoot, and secondly that there is an increased lateral bud development, leading to witches brooms.

Skoog and Tsui (1951) reported that increased KH_2PO_4 increased bud formation and quoted R. W. Howell as definitely ascribing this effect to H_2PO_4 radical. It would be interesting to see whether these sulpha drugs stimulate H_2PO_4 absorption.

It thus seems that a host of factors must be responsible for the profound effect of these sulpha drugs on sugarcane.

The sprouting of lateral buds and production of adventitious buds point to the possibility of increasing tillering by working out the proper drug and its concentration, time of application, etc.; for, if the lower buds are induced to sprout early in the life-cycle, they can avail of the advantage of the grand period of growth and may grow into mature canes, thereby holding out promise for increased yields.

As to the drugs themselves, an interesting correlation has been observed between the constituents of the side chain and the effects produced. Taking Sulphanilamide (I) and its two derivatives, *i.e.*, Sulphaguanidine (II) and Sulphathiazol (III).



I II III

into consideration it seems clearly to stand out that as the length of side chain increases the extent of disturbances produced decreases. Finally in Sulphathiazol a 5-membered ring also comes to be incorporated and this might explain the mild effect of this drug for, as a rule, 5-membered rings do not confer activity; however, the fusion of a 6-membered ring with a 5-membered ring is supposed to confer activity (Thimann, 1951).

Such being the profound effects of these sulpha drugs, further experiments in micro plots have been laid out and their results would form the subject-matter of a separate communication in due course.

V. SUMMARY

Influence of five sulpha drugs, *viz.*, Sulphanilamide, Sulphadiazine, Sulphaguanidine, Sulphamerazine, and Sulphathiazol has been studied under pot culture conditions.

Variations in growth exposition as influenced by these have been described with a critical discussion in the context of the latest available literature on the mechanism of these drugs.

Further possible lines of work have been put forward.

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Explanation of Plate XIX

Fig. 1. Showing linear chlorosis in leaf. Sulphathiazol—3 gm.

Fig. 2. Showing influence of drugs on root growth: (1) Control. (2) Sulphadiazine 1·5 gm. (3) Sulphadiazine 3 gm. (4) Sulphathiazol 1·5 gm. (5) Sulphathiazol 3 gm. (6) Sulphanilamide 1·5 gm. (7) Sulphanilamide 3 gm. (8) Sulphaguanidine 1·5 gm. (9) Sulphaguanidine 3 gm. (10) Sulphamerazine 1·5 gm. (11) Sulphamerazine 3 gm.

Fig. 3. Showing development of multiple buds on the lower nodes. Sulphadiazine—3 gm.