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Effect of Application of Insecticides on the HCN Content and Rhizosphere Microflora of Sorghum Plants

D. KANDASAMY[1], T. MARIMUTHU[2], G. OBLISAMI[3], and T. R. SUBRAMANIAM[4]

Studies were conducted to examine the influence of HCN content of root which was altered by the application of two systemic insecticides. There was no relationship between the HCN content of the roots and the microbial population except *Azotobacter* which showed an apparent negative relationship. An increase in the population of *Aspergillus* spp. was observed with a simultaneous reduction in the HCN level of the root. The application of insecticides caused a spectacular decrease in the HCN content of the shoot system.

The amount of hydrocyanic acid (HCN) occurring in varieties of sorghum varies with the change in the environment, such as weather, soil fertility, and kind and amount of fertilizers (Patel and Wright, 1958). However, there seems to be no report on the changes in the microbial population in the rhizosphere in relation to HCN content of sorghum plants as influenced by the application of granular insecticides. The present paper reports the changes in the HCN content of sorghum and the total microbial population and different genera of fungi occurring in the rhizosphere of sorghum due to the application of systemic insecticides.

MATERIAL AND METHODS

Sorghum plants (culture No. 669) were raised in rows at a rate of 2 plant per hill at the University Experimental Farm. When the plants were one week old, carbofuran 3% G (2,2- dimethyl 2,3-dihydrobenzofuranyl 1-7-N methyl carbamate) at 4.5 g/meter and phorate 10% G (0,0-diethyl S-(ethyl thio)methyl phosphorodithioate) at 1.5 g/meter were applied around the roots. The root and shoot samples were analysed for the HCN content after 24hr, 3rd, 7th and 20th days of application of insecticides.

The method of HCN estimation was similar to that of Rangaswami and Balasubramanian (1963 a) with a modification that the colour intensity was measured in an Erma colorimeter, type A-11 at 530 nm against reagent blank. The bacterial, fungal, actinomycete and *Azotobacter* populations were estimated in the rhizosphere soil sample employing serial dilution plate method at periodical interval, using soil extract, Martin's rose-bengal, Kuster's and Waksman 77 agar media, respectively.

The effect of cyanide (HCN) on the multiplication of *Azotobacter* was determined as per the procedure described below: waksman 77 medium (without $CaCo_3$) was dispensed in 50ml quantities in 250 ml Erlenmeyer flasks

Tamil Nadu Agriculturral University, Coimbatore - 641003

and sterilized at 20 psi for 20 min. Accurately weighed quantities of potassium cyanide dissolved in minimal quantity of double distilled water, passed through millipore membrane filter were added to each flask. The flasks were inoculated with 1 ml suspension of *Azotobacter chroococcum* (ca. 4x 10^5 cells/ml) and incubated at room temperature (28-30°c) on a rotary shaker and the growth rate was measured periodically employing Erma colorimeter at 530 nm,

RESULTS AND DISCUSSION

There was a general decrease in the HCN content of control sorghum plant with increase in the age of the plant (Table I) which is in conformity with the observations of earlier workers (Nelson 1953; Mahudeswaran *et al*., 1958). Timonin (1942) while examining the relationship between the HCN content of roots of resistant and susceptible varieties of flax to wilt disease and the bacterial population found that the roots of resistant varieties contained higher quantites of HCN with a decreased bacterial population. However, the present study indicated the absence of any such relationship between the HCN content of roots and the microbial population except for *Azotobacter*. Application of phorate reduced the HCN level in the root on most of sampling periods with a concomitant reduction in the bacterial and actinomycete population. Contrarily, carbofuran which had increased the HCN level on the 20th day of sampling reduced the bacterial and actinomycete population (Table II). Rangaswami and Balasubramanian (1963 a) also reported no define relationship between the HCN content of the root and microbial population.

It is also interesting to note that the fungal population particularly *Aspergillus* sp. was more due to the application of the two insecticides (Table III), especially on the 7th and 20th day by phorate when the

TABLE. I HCN content in the root and shoot system of sorghum as influenced by application of granular insectcides

(mg/100 g of dry tissue)

Period of sampling	Plant parts	Control	Carbofuran	Phorate
24 hr	Root	24.139	13.655	6.205
	Shoot	98.085	58.057	52.444
3rd day	Root	29.015	27.372	30.109
	Shoot	107.160	80.370	76.250
7th day	Root	21.907	0.986	1.578
	Shoot	69.519	64.120	65.795
20th day	Root	11.194	21.268	3.917
	Shoot	71.411	61.081	40.540

TABLE II. Changes in the rhizosphere microflora of sorghum due to application of granular insecticides

Period of sampling	Microorganism	Control	Carbofuran	Phorate
	Bacteria	11.57	11.35	10.11
	Fungi	2.39	1.38	1.08
24 hr.	Actinomycetes	5.95	3.26	3.43
	Azotobacter	13.63	14.90	18.57
	Bacteria	5.26	13.03	10.11
3rd day	Fungi	4.15	4.97	1.75
	Actinomycetes	1.18	1.40	1.46
	Azotobacter	7.30	16.70	7.23
	Bacteria	35.73	24.63	25.83
7th day	Fungi	1.44	1.66	1.95
	Actinomocetes	2.38	1.22	1.10
	Azotobacter	10.80	24.20	20.10
	Bacteria	11.58	6.02	4.70
	Fungi	0.48	2.46	0.66
20th day	Actinomycetes	0.95	0.73	0 90
	Azotobacter	11.26	5.59	13.35

* bacteria - x 10^6 per g of moisture free soil; fungi - x 10^4 per g of moisture free soil; actinomycetes- x 10^5 per g of moisture free soil; Azotobacter X 10^5 per g of moisture free soil

HCN level in general, was low. Similarly, application of carbofuran increased the HCN level on the 20th day with a simultaneous reduction in the *Aspergillus* population. Rangaswami and Balasubramanian (1963 b) isolated *Aspergillus niger* from the rhizosphere of sorghum which was capable of utilizing potassium cyanide as sole source of N for its growth and indicated that the fungus might utilize and break down the HCN extracted from the roots, thus minimising its toxic effect.

Azotobacter population showed a negative relationship with HCN level in the root system. For example, the two insecticides decreased the HCN level of the root after 24 hr and 7th day of application of insecticides with a simultaneous increase in the *Azotobacter* population. The *in vitro* study conducted also proved the inhibitory effect of HCN on the *Azotobacter* population (Table IV).

Interestingly enough, the sorghum plants which received the application of granular insecticides registered a remarkably low amount of HCN in their shoot systems which suggests that these insecticides besides protecting the crop from the pest attack, may also eliminate the danger of poisoning of

TABLE-III. Changes in the fungal flora of rhizosphere soil of sorghum plant due to application of insecticides

(Data represent percentage of the organism over the total population examined under each treatment)

Period of sampling	Fungal genera	Control	Carbofuran	Phorate
3rd day	*Penicillium*	54.70	29.00	21.11
	Aspergillus	15.88	18.00	20.00
	Rhizopus	7.84	20.00	22.22
	Mucor	5.88	13.00	4.44
	Curvularia	1.96	—	2.22
	Fusarium	—	—	—
	Helminthosporium	—	—	—
	Others	11.50	20.00	20.00
7th day	*Penicillium*	48.94	20.45	26.15
	Aspergillus	20.25	41.81	45.37
	Rhizopus	12.82	6.81	5.77
	Mucor	5.12	18.18	7.70
	Curvularia	2.52	4.54	—
	Fusarium	—	—	—
	Helminthosporium	—	—	—
	Others	10.25	7.18	15.00
20th day	*Penicillium*	45.00	24.58	9.40
	Aspergillus	20.00	12.25	55.97
	Rhizopus	13.00	20.66	11.97
	Mucor	2.00	22.50	5.97
	Curvularia	1.00	2.08	—
	Fusarium	—	—	—
	Helminthosporium	—	—	—
	Others	19.00	17.72	15.82

TABLE IV Influence of potassium cyanide (KCN) on the growth of *Azetobacter chroococcum*

KCN Conc.*	Period of incubation			
	24 hr	48 hr	72 hr	96 hr
Control	0.015	0.02	0.03	0 035
5 mg.	0.01	0.015	0.015	0.02
10 mg.	0.005	0.005	0.005	0.01
25 mg.	0.015	0.005	0.01	0.01
50 mg.	0.015	0.01	0.01	0.015

* Per 50 ml of Waksman '77 medium (without calcium carbonate)
Figures indicate the optical density values.

HCN to livestock. The present study also indicated that while one specific group of microflora in the rhizosphere is encouraged by the presence of HCN, another group is suppressed indicating variation in their sensitivity to HCN, which quality depends again on the microecological niche of rhizosphere region.

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