SUITABILITY OF EXPERIMENTAL DIETS FOR EARTHWORM CULTURE

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SINCE Darwin focussed the attention of the scientific world on the earthworms through his famous book *Formations of Vegetable Mould through the Action of Worms, with Observations on Their Habits*[1] a good many investigators have carried out research work on one or the other aspect of these interesting invertebrates which reside in practically all soils of the globe. For over a decade from late forties the author, and his colleagues, had contributed to earthworm literature by furnishing information not only on the microflora associated with their alimentary canal[2] and the role they have in agriculture[3] with particular reference to the alteration they bring about on the microflora of soils[4], but also by indicating the manner in which the worms bring about azotobacterization of soils[5]. However, no systematic efforts were made during the first decade of work to ascertain which diet(s) would suit most the requirements of their growth and reproduction or from the point of view of dissemination of *Azotobacter* which was shown to be associated with their alimentary canal as well as their castings. The purpose of this communication is to present the results of investigations carried out by the author during the second decade but withheld from publication as the author had not during the time in his collection more than one variety of earthworms for experimentation. Since then experiments upon other varieties have been conducted and confirmatory evidence obtained on the species studied earlier.

In the earlier studies[6] the practice adopted for culturing of the earthworms was on *ad hoc* basis of blending of soil with vegetable wastes including weeds, and animal waste (compost, mainly animal dung) in the proportion 8 : 2 : 2. Sometimes straw was also used. No systematic efforts were made to establish the superiority or otherwise of any one item over the other, or advantages to be derived by mixing of different dietary materials ascertained. In the experiments reported below a slightly alkaline soil was first chosen as the preliminary experiments revealed sudden drops in pH in the experimental diet in which the earthworms were reared. Secondly, since at the time, use of ammonium sulphate was commended as a fertilizer, a set of experiments were also carried out by incorporation thereof in soil, although it was obvious that earthworms fed upon organic matter and would not make use of the mineral for their growth.

The experimental diets were made by mixing weighed quantities of red soil of pH 8·00 kept in mudpots (commonly used for growing garden plants) and incorporating therein on 10 : 1 dry weight basis cow dung, kitchen waste, green (leaf) manure, straw and a mixture of all the four above, and including ammonium sulphate. Ammonium sulphate was incorporated at the level recommended in soil as fertilizer. After blending, adult earthworms, in numbers specified in Table I, were introduced in the experimental diets and stabilization of conditions allowed to continue for 4 to 5 days, (but never over a week), and then, every two weeks, the earthworm populations were counted by hand picking. Microbiological analysis was limited to the use of modified Ashby's agar on which very accurate counts of *Azotobacter* (referred to as black colour colonies) could be made and which also permitted the growth of some other bacteria referred to in tables as colourless colonies. The significance of the latter remains to be ascertained. The microbiological analysis was not carried out after 4 weeks whereas earthworm populations were estimated upto the end of 7 weeks. The results of growth experiments in the experimental diets are presented in Table I.

From a glance at the table, it would be seen that the worms found the experimental diets 3, 4, 5, 6 and 7 as satisfactory as within 14 days adequate increase in progeny had taken place. Twenty-eight days utilization of the diet clearly showed that the worms perished in the presence of ammonium sulphate, (diet 2) whereas in others they multiplied reasonably well. Reproduction in the cow dung diet, green manure diet, and the straw diet was conspicuous. The parent progeny ratio, in fact, showed green manure and cow dung as superior to others, the ratio respectively for cow dung, kitchen waste, green manure and straw being 1 : 6, 1 : 3, 1 : 7 and 1 : 5·5. Closer examination of the results however shows that in the cow dung no cocoons were encountered, though 1 : 6 parent-progeny ratio was registered, whereas in others, cocoons were seen. Does this mean that cattle dung, being superior as will be clear from Table II permitted hatching expeditiously of the eggs ? The answer seems to be yes from the fact that at no time the

TABLE I

Earthworm population in soils

Sl. No.	Experimental Diets	Initial	After 14 days			After 28 days			After 47 days		
			A	Y	C	A	Y	C	A	Y	C
1.	Control Soil (CS)	6	6	..	..	6	..	..	4	10	..
2.	CS + $(NH_4)_2SO_4$	8	8	1	..	..	..	..	..	..	..
3.	CS + Cow dung	10	10	2	..	10	61	..	12	126	..
4.	CS + Kitchen Waste	8	8	6	..	9	21	1	9	38	..
5.	CS + Green Manure	8	8	14	2	8	53	5	11	110	..
6.	CS + Straw	14	14	4	..	15	72	4	15	10	..
7.	CS + 2—6	16	16	9	..	16	37	..	16	38	..

A = Adult worms; Y = Young ones; C = Cocoons;

cocoons were encountered within 47 days in cow dung as well as in the green manure though in the latter they were there earlier ; the young ones also attained maturity quickly and the population doubled itself. Straw feed, on the other hand, was unsuitable as the young worms died. Kitchen waste proved to be quite suitable whereas the mixture clearly was not so. The parent progeny ratio at this stage changed to 1 : 13, 1 : 5, 1 : 14, and 1 : 1 respectively. In the mixed diet, the ratio was at both stages 1 : 2, with approximation. Lack of food appears to have resulted in the death of two adults from the control though they seemed to have propagated to an extent.

A conclusion of greatest significance drawn from this experiment is that ammonium sulphate is highly detrimental to earthworm population and should never be used where earthworm culture is desired. In fact Edwards and Lofty[7] conclude in their recent book that "There is good evidence that sulphate of ammonia is antagonistic to earthworm populations".

Results presented in Table II bring out clearly, the extent to which the experimental diets of the worms can effect the nature of the bacterial flora associated with them. Evidentially, only 3 of the 6 diets tried were observed to be suitable from the point of view of promoting azotobacterial growth, the population of which is important from the view-point of biological nitrogen fixation. Of these, straw seemed to be the medium of choice for their propagation. That addition of straw in soil did not adversely affect the overall nitrogen status, though brought about losses in nitrate nitrogen, was shown long ago by Murray[8]. Desai[9] had even demonstrated nitrogen fixation under favourable conditions in soil in the presence of straw or organic matter. Likewise, Palacios and Bhat[10] also concluded from their studies on the effect of cellulose on the nitrogen status of soil exposed to different conditions of light and humidity that the presence of cellulose contributed favourably to its nitrogen status and that even after exposure of the soil to direct sunlight for 60 days in its dry state, the *Azotobacter* continued to live in soil. In other

TABLE II

*Bacterial counts/g of soil containing exptl. diets on modified Ashby's agar**

Experimental Diets	Initial**		After 14 days		After 28 days	
	B	C	B	C	B	C
1	..	700	..	1250	..	1700
2	..	500	..	900	..	1250
3	1850	500	2000	750	2150	1000
4	1150	400	1300	500	1400	500
5	..	1350	..	1700	..	2400
6	2550	450	3500	800	3700	900
7	..	450	..	1500	..	2450

* Described in ref. 6.
** After 5 days of stabilization on the introduction of the worms.
B = Black (*Azotobacter*) colonies.
C = Colourless colonies.

words, availability of suitable carbon source must have been responsible for the *Azotobacter* to flourish in straw containing soil, the earthworms no doubt contributing, in their singular way, to its population. It may however be recalled, that in the presence of straw alone, earthworms could not reproduce beyond 30 odd days and, what is worse, young ones could not thrive much longer for reasons unascertained so far.

Next to the straw diet, cow dung and kitchen waste, in that order, appeared to be suitable for the *Azotobacter* to flourish. Surprisingly, the green manure, which proved to be superior for the rapid reproduction of the worms, failed to support the growth of *Azotobacter*. This, in fact, was the reason for considering above cow dung as superior to green manure for the rearing of the earthworms. The possibility that, if the experiments were continued beyond the period arbitrarily fixed, the earthworms population in cow dung would perhaps have exceeded that encountered in green manure, was indirectly evidenced from the observation that at no time cocoons were met with in the cow dung culture, whereas in the green manure culture, they were present at both the times of examination. Besides, the worms growing in cow dung appeared less immature and more alert; also, elicited better response than those reared in green manure to stimuli, *e.g.*, touch; it is also interesting to note that the total number of bacteria growing on Ashby's medium recorded a higher count for the cow dung soil than did the kitchen waste soil, not to point out the relatively steady increase encountered in the *Azotobacter* count in the former.

That cow dung serves adequately the purpose of earthworms cultivation has been pointed out by Edwards and Lofty[7]. It was of interest to ascertain why the earthworm populations tended to dwindle or disappear in some of the diets. Determination of pH of the diets was considered in this context of holding the clue. Accordingly, pH at two stages, indicated in Table III, was measured with glass electrode. The results revealed the conspicuous and gradual fall in the ammonium sulphate soil and to a lesser extent in the soil containing the mixed diet. Wherever earthworms flourished, there was a tendency for the pH to rise after an initial fall, except in ammonium sulphate soil wherein the pH continued to fall and earthworm got killed at about pH 5·0 Kitchen waste also, at initial stage, lowered the pH and in all probability the rising earthworm population tended to counter such changes. In the mixed diet the ammonium sulphate, on the other hand, had its drastic effect, as even after 47 days, there was no indication of any young worm attaining adulthood as witnessed with other diets.

TABLE III

pH of soils containing experimental diets and worms

Experimental Diets	Initial	After 14 days	After 28 days	After 42 days
1	8·0	8·15	8·0	7·85
2	6·2	5·3	4·9	4·55
3	8.15	7·9	7·9	8·00
4	7·8	7·4	7·35	7·5
5	7·9	7·8	7·8	7·95
6	8·0	7·9	7·9	7·95
7	6·55	7·1	5·85	5·3

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