INVESTIGATIONS ON THE RÔLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part V. Influence of Minute Quantities of Certain Forms of Organic Matter on the Growth of Barley.

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ALTHOUGH it is now definitely established that the plant depends on the soil for its supply of minerals and, in most cases, also for its nitrogen, the extent of its dependence for organic matter is still comparatively obscure. There has, nevertheless, always been a school of thought contending that the humic matter of the soil or the organic matter added in the form of manures provide certain substances which are essential to plant growth. This view has found favour in practical farming and, indeed, progressive farmers of all times have attached considerable importance to humus as plant food. Grandeau (1872) was probably the first to adduce scientific evidence to show that the organic matter of the soil may supply a part of the carbon requirements of the plant. A number of later workers have arrived at similar conclusions, though quantitative data regarding the extent of indebtedness of the plant to the soil are still wanting.

There is considerable amount of evidence to show that even on soils which are fairly rich in humus, organic manures yield better and more consistent results than mineral fertilisers. As instances of this, may be cited the observations on the permanent manurial plots at Rothamsted and Woburn (Russell, 1926). The effect is still more pronounced on tropical soils which are naturally deficient in organic matter and on which mineral fertilisers either fail to evoke the same response as in temperate regions, or, sometimes, even depress the yield. The superiority of organic manures over the commoner forms of mineral fertilisers may be due to one or more of the following :—They may (*a*) provide minute quantities of certain rare, but nevertheless essential, inorganic constituents not ordinarily present in the latter ; (*b*) enrich the atmosphere around the plant with carbon dioxide and thus facilitate increased assimilation ; or (*c*) supply certain organic substances, which would serve as accessories to plant growth. Only scanty information regarding (*a*) is available, while there is extensive literature relating to (*b*) and (*c*). Some of the related evidence has been critically examined in a previous communication (Subrahmanyan and Siddappa, 1933[1]). Further material will be considered in the present paper.

There is apparently much of contradictory evidence regarding the rôle of organic matter as a source of carbon dioxide. The direct experiments conducted with that gas would point to its being useful under certain conditions, but useless and even inimical to plant growth under others (Lundegårdh, 1924, 1927, 1928; Keuhl, 1925; Reinau, 1926; Rippel, 1926; Gerlach, 1926; Bolas and Henderson, 1928; Hasse and Kirchmeyer, 1928; Small and White, 1930; White 1930 and several others). A critical study of the related literature would show that the conditions were not always favourable to assimilation. In some cases, root aeration would appear to have been impaired. The more recent observations of Subrahmanyan and Siddappa (1933[2]) would show that, at any rate in the early stages, carbon dioxide formed through oxidation of organic matter in the soil is sufficient to meet all the requirements of plant life.

The literature relating to the production of accessories to plant growth is highly controversial and, in places, even contradictory, but the general evidence would point to the existence of certain substances, which though not absolutely essential (in the sense that vitamins are), are nevertheless helpful to general growth and reproduction (Schreiner and Reed, 1907; Schreiner and Shorey, 1910; Bottomley, 1914, 1915, 1917, 1919, 1920; Mockeridge, 1915–1917, 1920, 1924; Itano, 1923; Hunter, 1923; Clark, 1924; Wolfe, 1926; Viswanath and Suryanarayana, 1927, 1934; Ashby, 1928, 1929; Olsen, 1930; Virtanen and Hausen, 1933, 1934; Subrahmanyan and Siddappa, 1933; Nicols, 1934). The mechanism of the action of such substances is still obscure. Since all soils contain organic matter, though in varying quantities, the practical significance of these auxiliary bodies in field practice is uncertain. Indeed, some of the preparations containing such substances (*e.g.*, bacterised peat) proved comparative failures when tried on the field.

The study of growth-promoting factors in plants is closely allied to similar enquiries relating to microorganisms (Wildiers, 1901; Fulmer, Nelson and Sherwood, 1921; Nelson, Fulmer and Cessna, 1921; Robertson, 1921; Funk and Dubin, 1921; Heller, 1922; Robbins, 1922; Macdonald and McCallum, 1921; Macdonald, 1923; Tanner, 1924; Devereux and Tanner, 1927; Werkman, 1927; Narayan, 1930; Farries and Bell, 1930; Fulmer, Williams and Werkman, 1931; Buston and Pramanik, 1931; Buston and Kasinathan, 1933; Nielson, 1930, 1931, 1934; Nielson and Hartelius, 1931, 1932, 1933; Elvetjem, 1931; Boysen Jensen, 1931; Schopmeyer and Fulmer, 1931; Burk, Lineweaver and Horner, 1932; Richards, 1932; Euler and Philipson, 1932; Philipson, 1933; Allison and Hoover, 1934; Hartelius, 1934, and several others). The latter would, in fact, appear to have a direct bearing on plant nutrition since (*a*) decomposition of organic matter in the soil proceeds largely through the agency of microorganisms, and (*b*) some of the products of microbial metabolism have been reported to act as stimulants to plant growth and reproduction.

In spite of these useful advances, the precise mode of action of organic manures in field practice is still ill-defined. Although several workers have adduced evidence to show that under controlled conditions, even traces of certain forms of organic matter can produce striking effects on plant growth and reproduction, it has yet to be admitted that the same substances or preparations containing them have to be applied in very large quantities before any beneficial effect can be observed on the field. Indeed, it is well known that, as distinct from mineral fertilisers, organic manures are effective only when applied in bulk. This would suggest that the active substances are either largely destroyed or otherwise reduced in efficacy under field conditions. It is also probable that some of the effects can be observed only at certain stages in the life of the plant. In view of this and the need for further knowledge regarding the mode of action of organic matter in general, the present enquiry was undertaken.

Experimental.

Effect of minute quantities of certain forms of organic matter on barley. Preliminary experiments.—The pots were made up with sand which had been repeatedly washed with water. They were then treated as follows:—(*a*) dried blood, 0·05 g.; (*b*) dried blood, 0·1 g.; (*c*) pressed brewery yeast, 0·05 g.; (*d*) pressed brewery yeast, 0·1 g.; (*e*) farmyard manure, 0·05 g.; (*f*) farmyard manure, 0·1 g.; and (*g*) control (untreated). Five pots were allotted for each treatment. Graded and previously washed seeds (Var., Plumage Archer) were sown at the rate of 50 per pot. The seedlings were nursed in wire-gauze cages which were exposed to the atmospheric conditions, but were protected against strong wind and rain. Distilled water was used for watering. After one month, the seedlings were removed, washed free from adhering sand and their dry weights determined. The related data have been presented in Table I.

TABLE I.

Treatment	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
Dried blood (0·05 g.)	175	18·2	18·7	33·6
Do. (0·1 g.)	165	17·7	15·3	30·8
Pressed yeast (0·05 g.)	160	18·3	16·8	25·9
Do. (0·1 g.)	185	14·5	19·3	30·2
Farmyard manure (0·05 g.)	160	17·1	17·5	34·2
Do. (0·1 g.)	170	22·7	19·7	38·0
Control (untreated) ..	165	13·5	13·2	23·7

The best growths were obtained in the case of specimens treated with farmyard manure. Dried blood and yeast followed next in order. The untreated ones came last: they were in fact dying at the time of completing the experiment. These observations are generally in agreement with those of Viswanath and Suryanarayana (*loc. cit.*).

The foregoing experiments were somewhat defective as it was subsequently found that, even after repeated washing, the sand contained 0·2 per cent. of carbon, corresponding to about 0·35 per cent. of organic matter. Moreover, the substrate was deficient in mineral nutrients. It was difficult therefore to differentiate between the effects of the organic constituents present in the added substances and such minute quantities of minerals as may be released through their decomposition.

Trials on pure sand and with complete minerals.—The sand used in this and subsequent experiments was of select quality which was further washed repeatedly with concentrated hydrochloric acid until it was entirely free from organic matter. The minerals were added in the form of chemically pure substances—calcium acid phosphate (0·17 g. per pot), potassium sulphate (0·15 g.) and potassium nitrate (0·1 g.). The different organic substances were then added and the seedlings raised in the same manner as described in the previous experiment.

TABLE II.

Treatment	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
Pressed yeast (0·05 g.)	140	25·3	21·0	47·9
Do. (0·1 g.)	160	27·2	24·7	45·2
Farmyard manure (0·05 g.)	165	24·2	23·5	44·7
Do. (0·1 g.)	150	22·5	20·8	48·2
Dried blood (0·05 g.)	165	23·7	22·3	50·3
Do. (0·1 g)	165	20·7	20·7	42·5
Control (untreated) ..	175	20·2	18·7	32·9

It may be seen from the results (Table II) that the seedlings receiving minute quantities of different organic substances were consistently better than those raised on minerals alone. The latter were drying at the tips and were generally on the decline at the time of concluding the experiment.

The beneficial effects of different organic substances might have been due to (*a*) certain water soluble constituents which were readily taken up by plants, or (*b*) products of their decomposition by microorganisms. Some experiments were accordingly carried out to verify these two possibilities.

Response to dried blood both before and after decomposition.—The pots were prepared in the same manner as in the previous experiment. Basal dressings of minerals were also given. The pots were then divided into groups of twenty each and treated as follows:—(*a*) dried blood at 0·05 g. per pot and sown immediately with barley; (*b*) aqueous extract of dried blood corresponding to 0·05 g. and sown as in (*a*); (*c*) dried blood as in (*a*) but sown after one month; and (*d*) extract of dried blood as in (*b*) and sown as in (*c*). Corresponding controls (untreated) were also maintained. The seedlings were removed after one month and their dry weights determined.

The above experiments were carried out in two successive seasons of the year. In the first set, they were commenced in dry weather and ended when the South-West monsoon was just getting to be active. In the second, they were commenced in wet weather and concluded when it was comparatively dry. The results have been presented in Tables III (*a*) and III (*b*).

TABLE III (*a*).

Treatment	Sown immediately (21-5-1932)		Sown after one month (21-6-1932)	
	Number of seedlings	Average dry wt. (in mg.) per seedling	Number of seedlings	Average dry wt. (in mg.) per seedling
Dried blood (whole) ..	680	33·3	640	28·7
Do. (extract) ..	600	42·5	540	32·7
Control (untreated) ..	660	36·4	540	36·6

TABLE III (*b*).

Treatment	Sown immediately (25-8-1932)		Sown after 20 days (15-9-1932)	
	Number of seedlings	Average dry wt. (in mg.) per seedling	Number of seedlings	Average dry wt. (in mg.) per seedling
Dried blood (whole) ..	480	38·7	300	30·2
Do. (extract) ..	500	40·1	300	27·9
Control (untreated) ..	660	33·4	420	27·6

It may be noted that in both the sets of experiments, the aqueous extract of dried blood evoked even better response than the whole product. This would show that the improved growth was due to certain water-soluble constituents. On standing for some weeks, however, the active substances were obviously lost : the controls yielded nearly as good and, in one set, even better results than the treated ones.

Decomposition of the active substance. Production of ammonia.—Although the sand used in the previous experiment was practically free from living organisms and the seeds had been washed with antiseptic solution prior to sowing, it yet appeared probable that the microorganisms present in air or introduced through dust had acted on the active substances and destroyed them. Since dried blood is rich in nitrogen (12·5 per cent.), it appeared probable that the extent of decomposition would be indicated by the production of ammonia. Some experiments were accordingly carried out adding dried blood to sand and following the production of ammonia at convenient intervals. The estimations were carried out by extraction with saline followed by distillation with magnesia and Nesslerisation of the distillate.

The results, which have been presented in Fig. 1 would show that partial decomposition of dried blood had occurred during the period of observation, so that it is presumable that the active substance was lost along with it.

Bacterial numbers.—The platings were carried out according to Thornton (1922). The counts (Fig. 2) show that there was marked increase in both

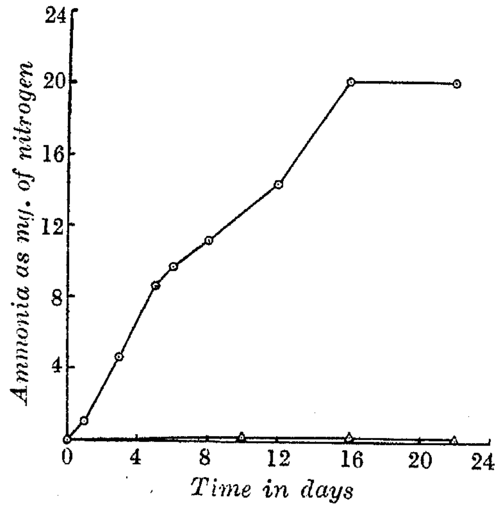

Fig. 1. Production of ammonia.

o—o Treated with dried blood (Experimental)
Δ—Δ Untreated (Control)

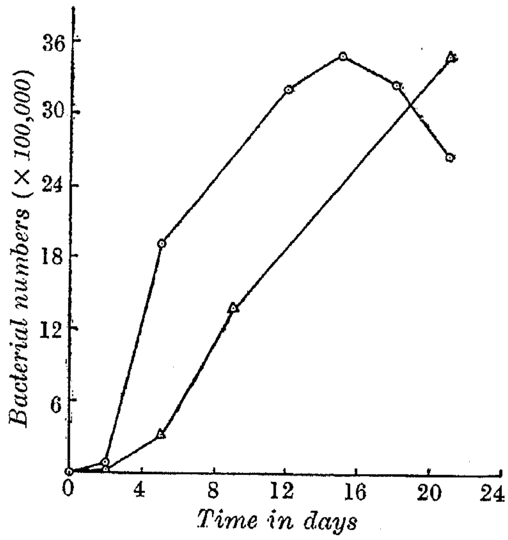

Fig. 2. Bacterial numbers.

o—o Treated with dried blood (Experimental)
Δ—Δ Untreated (Control)

the experimental and the control specimens. The rise was more rapid in the case of dried blood owing probably to the presence of readily available nutrients. The increase observed in the case of the controls was obviously due to the presence of ungerminated seeds, which underwent decomposition in the later stages. The observations would show that, however carefully prepared, a bed of sand cannot be maintained sterile over any great length of time. They also support the conclusion that the active substance was decomposed through microbial agency on prolonged standing.

Effect of dried blood extract at different stages.—The pots were prepared in the same manner as in the previous experiments and sowed with barley which had been previously steeped in water for 24 hrs. Immediately after sowing and at different stages after germination, the sand was treated with dilute extract of dried blood in quantities corresponding to 0·05 g. of solid per pot. The observations made at the end of three weeks have been recorded in Table IV.

TABLE IV.

Time of treatment (in days) after sowing	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
0 ..	90	29·8	33·6	58·2
5 ..	90	25·0	35·4	47·6
12 ..	120	22·4	33·4	44·5
15 ..	130	25·8	37·2	47·9
Sand+minerals (control)..	150	24·7	35·3	42·7
Sand+minerals+ash of dried blood (control) ..	125	24·2	36·2	44·2

The best results were obtained when the extract of dried blood was applied immediately after sowing. The effect is traceable to the better development of the shoot.

It was noted that although 250 seeds were sown for each trial, the number of seedlings found at the time of completing the experiment was comparatively small. This was first inexplicable, but subsequent observations showed that it was largely due to steeping in water prior to sowing. Similar results were also obtained by Ramachandra Rao and Subrahmanyan (1934), who showed that the adverse effect was due to inadequate supply of air. Treatment with dried blood was also found to be inimical to germination though it subsequently proved helpful to plants that came up. The observations made during two different seasons have been presented in Figs. 3 and 4.

Both dried blood and its extract were, to some extent, inimical to germination. The premonsoon period with its comparatively dry weather was more favourable to germination than the wet season that followed.

In view of the low germination, especially in presence of dried blood, it appeared probable that the better development of seedling observed in that case was more due to diminished competition than to the influence of any foreign, growth-promoting substance.

Effect of treatment with dried blood under controlled conditions of temperature and light intensity.—With a view to eliminating the above-

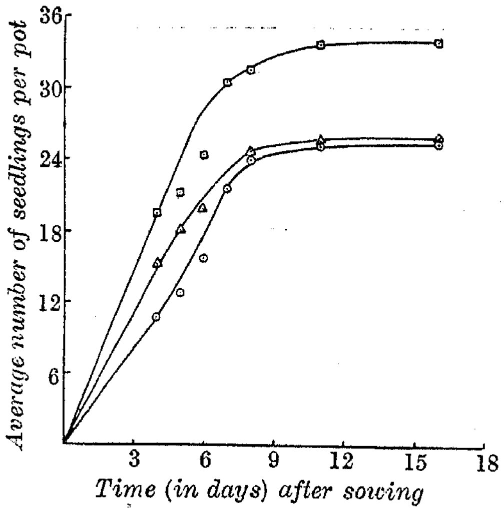

FIG. 3. Effect of steeping on germination of barley. (Premonsoon period)

o—o Sand + minerals only
o—o ,, + ,, + dried blood
△—△ ,, + ,, + extract of dried blood

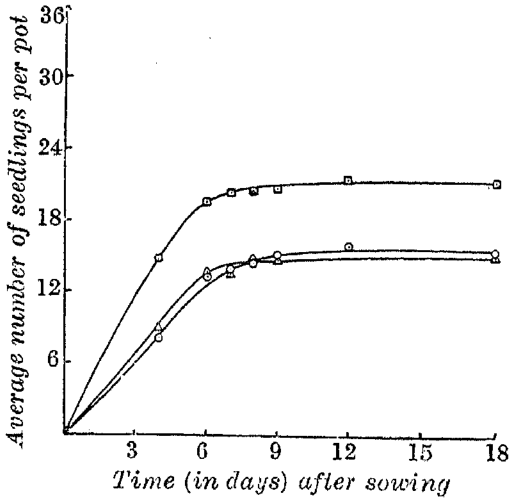

FIG. 4. Effect of steeping on germination of barley. (Monsoon period)

o—o Sand + minerals only
o—o ,, + ,, + dried blood
△—△ ,, + ,, + extract of dried blood

mentioned defects and to obtaining results that could be reproduced, the experiments were next repeated with the following modifications :— (*a*) In addition to complete minerals and ash of farmyard manure, ash of dried blood (corresponding to 0·05 g.) was also added as part of the basal treatment. (*b*) The small quantities of dried blood (0·05 g.) or equivalent amounts of extract were mixed with sufficient quantity of water to ensure uniform distribution over the surface of the sand. (*c*) Prior to sowing, the seeds were not steeped as in the previous experiments, but washed a number of times with distilled water. (*d*) The pots were kept in a room (20 ft. by 10 ft.) with thick walls (2 ft.) which was well insulated against heat and completely shielded against external light. The walls were painted dark. The room was maintained at constant temperature (24°C.) and illuminated artificially. The lighting arrangement consisted of a gas-filled lamp (500 c.p.) fixed on the ceiling, 15 ft. from the ground. Illumination was provided for 9 hrs. in the day, from 8 a.m. to 5 p.m. Adequate air supply was ensured by inlet and exhaust fans with down draught arrangement which were fixed at either end of the room. The pots were arranged in a square in the centre of the room, those receiving different treatments being distributed at random. In one set of experiments, the seeds were sown immediately after treatment, while, in the other, a period of 15 days was allowed to lapse after addition of dried blood. The results have been presented in Table V.

TABLE V.

Treatment	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
Dried blood (0·05 g.)—Sown immediately	322	31·0	18·3	26·4±2·1
Extract of dried blood—Sown immediately	308	32·3	18·4	27·7±2·0
Dried blood (0·05 g.)—Sown after 15 days	329	29·7	18·7	26·3±0·9
Extract of dried blood—Sown after 15 days	315	31·1	13·3	29·4±2·6
Control (untreated)—Sown immediately	322	31·3	18·1	27·4±2·3

There was satisfactory germination (about 90 per cent.) in all the cases. As compared with those in the previous experiments, the seedlings grew up very tall and thin. This was due to the intense artificial illumination (Varadachar, 1933). The seedlings were rich and juicy, but their dry weights were comparatively small. It is probable that the long period (15 hours) of darkness following the daily illumination was responsible for profuse respiration and consequent loss in weight. Competition among seedlings may also partly account for that observation. The average dry weights of seedlings did not appreciably differ from each other. The observations would indeed suggest that under the conditions of the above experiment, minute quantities of dried blood, either as such or as aqueous extracts, had no effect on the growth of barley.

Effect of higher concentration and better distribution of dried blood.—The quantities of dried blood used in the previous experiments were so small that it appeared probable that some of the effects were due to uneven distribution of solid matter. With a view to verifying this and to determining the response to varying concentrations of dried blood, the following experiments were carried out. Solid dried blood was thoroughly mixed with sand and varying quantities of the mixed product spread uniformly over the surface. When working with the aqueous extract, the volumes were so adjusted that the liquid, which was introduced drop by drop, could moisten the entire surface. The sand was then turned over repeatedly until a homogeneous mixture was obtained. The other details were the same as in the earlier experiments except that the seeds were sown without previous steeping.

TABLE VI.

Treatment	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
Dried blood (0·05 g.)	235	19·5	23·8	33·9±4·4
Do. (0·2 g.)	240	19·0	23·1	33·6±4·4
Do. (0·5 g.)	225	14·2	21·3	30·9±3·4
Extract of dried blood from (0·05 g.)	235	13·3	25·8	27·8±2·7
Do. from (0·2 g.)	230	13·3	21·7	33·4±2·8
Do. from (0·5 g.)	230	14·8	23·1	32·5±3·5
Ash of dried blood (0·2 g.)	225	16·2	22·4	31·3±4·0

It may be seen from the results (Table VI), that when unsteeped seed was used, the difference between the various treatments practically disappeared. It is probable that larger doses of dried blood may prove beneficial in the later stages of plant life, but so far as seedlings are concerned, the effect is about the same in all the cases.

Effects of minute quantities of yeast and dried blood at different stages of plant growth.—Although the foregoing observations suggested that (at any rate in sand-cultures) minute quantities of different organic substances had comparatively little effect on the growth of seedlings, it still appeared probable that certain beneficial effects may be produced in the later stages. Some experiments were conducted, therefore, growing the plants to maturity. Fairly big-sized pots were made up entirely with acid-washed sand (free from organic matter) and treated with complete minerals in the same proportions as in the previous experiments. The seeds were sown at the rate of six per pot, but as the seedlings came up, they were reduced to two each. Ungerminated seeds, as also traces of adhering organic matter, were carefully removed. The pots were all kept in wire-gauze cages which were shielded against dust and watered with distilled water.

The pots were divided into batches of five each and treated as follows:—(*a*) pressed yeast (dried), 0·1 g.; (*b*) aqueous extract of 0·1 g. of yeast; (*c*) ash of 0·1 g. of yeast; (*d*) dried blood, 0·1 g.; (*e*) aqueous extract of 0·1 g. of dried blood; (*f*) ash of 0·1 g. of dried blood; and (*g*) control (untreated). The aqueous extract of yeast or dried blood was prepared by shaking 8 g. of the dry powder with 320 c.c. of distilled water in a reciprocating shaker for 30 mins. and then filtering. The filtrate was examined microscopically in each case to ensure the absence of living organisms. The extract obtained from yeast contained 0·23 per cent. of solids and that from dried blood, 0·11 per cent. The experimental pots (groups *b* and *d*) were treated with 4 c.c. each of either extract.

It was observed that although the seedlings came up quite well in all the cases, the growths were not sustained in the later stages. Although the plants continued apparently healthy, the general development was so poor that none of them grew up to be more than a foot in height. In many cases, the tips showed signs of drying prematurely. Tillering was very poor. Flowering and grain formation were also disappointing. Some of the plants bore no grains at all, while the others yielded only two or three each. In view of these unsatisfactory results, the quantitative data have not been recorded.

The poor plant growth may have been due to one or more of the following causes :—(*a*) absence of sufficient quantities of organic matter ; (*b*) inadequate supply of certain essential mineral nutrients ; (*c*) presence of toxic substances. Both (*a*) and (*b*) are probable, but it is hardly likely that any toxic substance was present. The sand had been repeatedly washed with water until it was chloride-free and then spread out to dry for a number of days, so the adverse effect could not have been due to the presence of even traces of hydrochloric acid. Watering was also done with care, so there was no danger of water-logging the roots : nor was there any possibility of loss of nutrients through profuse drainage since the quantities of water that were added were just sufficient to make up for loss by evaporation.

Response to minute quantities of farmyard manure in presence of a culture solution.—With a view to avoiding the possible defects in the previous experiments, the following modifications were made : (*a*) the inorganic salts that were applied as part of the basal treatment were replaced by Meyer's culture solution (1932) which includes sodium, iron, magnesium and chloride in addition to the usual nitrogen, potassium, phosphorus and calcium ; (*b*) the minerals were supplemented by the product (2·0 g.) obtained by treating soil repeatedly with hydrogen peroxide (beginning with 6 per cent. and increasing to 30). It was hoped that this treatment would leave the nutrients in a more readily available form than the drastic one of ignition.

To pots containing 250 g. each of acid-washed sand, 4 c.c. each of Meyer's solutions (A) and (B) were added followed by application of peroxide-treated soil which was spread uniformly throughout the surface. The following were the treatments: (*a*) farmyard manure at 0·05 g. per pot; (*b*) product of treating farmyard manure (0·05 g.) repeatedly with hydrogen peroxide and then evaporating the resulting suspension. The seedlings were raised in the same manner as described in some of the previous experiments.

TABLE VII.

Treatment	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
Farmyard manure (0·05 g.)	240	16·5	10·5	32·8±1·3
Product of treating farmyard manure (0·05 g.) with H_2O_2	240	15·4	9·2	33·0±2·8

The results (Table VII) show that when all the essential mineral nutrients were provided, addition of minute quantities of organic matter made no appreciable difference to growth.

Although Robinson (1927) has shown that certain forms of organic matter present in or associated with the soil are resistant to the action of hydrogen peroxide, it was yet found that the oxidation was complete in the case of the soil or farmyard manure used in the present study. The treated specimens did not contain any lime, chromates or manganese dioxide and this was probably largely contributory to the efficiency of the oxidation.

Influence of change of substrate on the growth of barley.—In the foregoing experiments all the conditions had been altered with the exception of the substrate. Although evidence has already been adduced to show that no toxic substance could be present in acid-washed sand, yet it appeared probable that sand may not be a suitable medium for bringing out the effects of minute quantities of organic matter. It was considered desirable, therefore, to try another substrate, which, while making a suitable medium for plant growth, would yet be free from carbon compounds. After trying a number of substances such as asbestos, kaolin and kieselguhr, it was found that laterite was eminently suitable for the purpose. Soil treated repeatedly with hydrogen peroxide was also found to be useful, but since large quantities of the reagent were required for obtaining even a few pounds of the product, the experiments were carried out only with laterite.

The type of laterite occurring in Bangalore and Kolar districts and, indeed in many parts of the Deccan plateau, contain only minute quantities of organic matter. In certain parts, especially where there is no vegetation and the surface is continuously exposed to weathering, it forms hard lumps which contain iron and alumina together with some quantities of silicates, but practically no carbonaceous matter. Even the traces that are present occur largely at the surface and can be easily washed off with water. Big lumps of this type of laterite (which is rather different from that found in other parts of the country where the rainfall is much heavier) were quarried from the Institute area, broken up to the desired size and then repeatedly washed with water. The product thus obtained was completely free from carbon as verified by wet combustion.

The pots were made up with laterite in the usual way and after adding (1) 5 c.c. each of Meyer's solutions A and B, and (2) the product obtained by treating soil (80 g.) with hydrogen peroxide, they were divided into batches of six each and treated as follows:—(*a*) farmyard manure (0·05 g.); (*b*) ash of farmyard manure (0·05 g.); (*c*) dried blood (0·05 g.); and (*d*) ash of dried blood (0·05 g.). The other details were the same as those of the previous experiments.

TABLE VIII.

Treatment	Number of seedlings	Averages		
		Shoot height in cm.	Root length in cm.	Dry weight (in mg.) per seedling
Farmyard manure	264	20·2	7·2	32·3±3·1
Ash of farmyard manure ..	264	17·0	6·0	32·4±2·4
Dried blood	270	15·5	5·3	29·6±1·7
Ash of dried blood ..	258	17·6	6·8	37·1±6·4

It may be seen from the results (Table VIII) that although the dry weights had not appreciably altered, the root lengths were very much less than those observed in some of the previous experiments (*cf.* Table VI). This was obviously a feature of growth on laterite and might have been due to either the aggregation of particles and general hardening in the lower strata, formation of toxic substances or development of other undesirable conditions below the first few inches. The seedlings raised on ash of dried blood had the biggest average dry weight, but the attendant error was so large that it cannot be regarded as being significantly different from the others. The results would indeed show that when all the requisite minerals are provided, minute quantities of the organic matter in either farmyard manure or dried blood make no perceptible difference to the growth of seedlings.

Further experiments with the different fractions of farmyard manure.—Since the previous observations had shown that the results obtained on laterite were, on the whole, similar to those on acid-washed sand, the present set of experiments was carried out with the latter. The trials were designed so as to bring out the effects of (*a*) mineral fertilisers, (*b*) the organic as well as the inorganic constituents of soil; (*c*) mineral matter in farmyard manure as obtained by different methods; and (*d*) the water soluble as well as the water insoluble constituents of farmyard manure—on the growth of barley. After being made up with sand, the pots were divided into groups which received the following basal treatments:—(A) sand + complete minerals as in the earlier experiments (control); (B) as in A + soil (80 g.) treated with

TABLE IX.

Treatment	Number of seedlings	Averages		
		Shoot length in cm.	Root length in cm.	Dry weight (in mg.) per seedling
A (control)	114	21·6	20·4	39·7± 6·5
B alone	255	23·3	20·3	36·2± 1·8
B+Ash of Fy.M. (0·05 g.) ..	245	23·4	20·3	43·2±12·7
B+Fy.M. treated with H_2O_2	237	28·4	24·5	42·0± 3·9
B+Fy.M. (0·05 g.) .	288	23·5	20·8	35·4± 4·2
B+Aqueous extract of Fy.M.	221	24·8	24·8	38·8± 6·0
B+residue after extracting Fy.M.	171	25·2	26·0	36·8± 2·9
C alone	290	26·9	22·3	42·6± 5·3
C+Fy.M. treated with H_2O_2	291	25·0	22·4	37·5± 5·0
C+Ash of Fy.M. ..	290	24·5	21·6	36·8± 3·7
D alone	269	23·0	25·1	35·0± 3·4
D+Ash of Fy.M. ..	257	25·3	27·4	37·7± 2·3
D+Fy. M. (0·05 g.) ..	263	23·6	23·6	35·8± 4·4

hydrogen peroxide; (C) as in B but with the difference that the minerals were applied in stages; and (D) as in A + fresh soil in quantities corresponding to that in B. The groups were further sub-divided and treated in the manner shown in Table IX. For each such treatment 10 pots were generally allotted though, in a few cases, only 8 or 6 were employed.

It may be seen from the results that there is no significant difference between the control and the various treatments.

Discussion.

The results of the present enquiry have brought to light certain facts of considerable scientific interest. They have also raised fresh problems of much practical significance.

Although organic manures are highly effective in field practice (especially under tropical conditions) their action is, nevertheless, insignificant when applied in minute quantities to sand-cultured plants which are provided with complete minerals. While not eliminating the possibility of the manures containing some growth-promoting substances, the observations would show that, under the conditions of the present study, such substances were either decomposed or otherwise rendered ineffective. There was indeed evidence to show that even when pure, inorganic substances were used and the experiments conducted under dust-free conditions, there was marked increase in bacteria and attendant decomposition of the added organic matter.

If it be admitted that the active substances were lost during the decomposition, the same argument would apply in much greater measure when considering their potency in the soil. Microbial decomposition proceeds very rapidly under field conditions, so that the practical significance of adding minute quantities of even the most active forms of growth-promoting substances would appear to be doubtful.

It may be reasonably expected that extract of dried blood or farmyard manure would have led to improved plant growth if applied in fairly large quantities and in repeated doses. This would, however, only complicate the issue since it would not be possible to distinguish between the effect of the growth-promoting substances (which would be required only in traces) and that of the different products of microbial decomposition.

Taking all known facts into consideration, the results of the present enquiry would lead to the conclusion that even if the organic manures contain growth-promoting substances which act in water cultures and other specialised conditions, they are yet of no practical significance in field practice. Organic manures act only in bulk, so their influence on plant growth would appear to be in other directions.

It may be argued that the present study was carried out with only one crop and that in most of the experiments the observations were not extended beyond the seedling stage. To that, it may be pointed out that if the growth-promoting substance does exist and is potent, it should produce the desired effect on any plant. Most of the previous trials were carried out with aquatic plants which have only some academic interest. If the effect is of any practical significance, it should be prominently seen in the case of agricultural crops, especially those of some economic importance. As for the trials being confined to seedlings, it may be stated that there is considerable practical difficulty in extending the observations beyond the seedling stage. In addition to the cost and labour involved in growing an organic material (the plant) in inorganic surroundings, it would be found to be almost impossible to keep out dust and avoid microbial action over any great length of time. Attention may also be drawn to the experiments in which attempts were made to grow the plants to maturity on acid-washed sand and exclusively on mineral nutrients. Under such conditions, the plants made no useful headway and the results were discordant, so it has to be inferred that, by prolonging the period, the object of the experiment would be defeated.

Many of the previous investigators who observed response to minute quantities of different organic substances had worked with water cultures. Their observations would suggest that the active substances were more or less stable under such conditions. Water cultures have only restricted interest, so if the beneficial effects are to be obtained under field conditions, the extracts containing the active substances should be fed directly to the plant. The possibilities of the injection method in this direction have already been indicated by Subrahmanyan and Varadachar (1933). The technique has since been extended to a number of plant species with satisfactory results. A systematic study of the effect of injecting different organic extracts at various stages of plant life was therefore carried out. The results of that enquiry which are of much interest will be dealt with in the next communication.

Summary.

1. When barley seeds which had been previously steeped in water were sown on partially washed or acid-washed sand treated with mineral fertilisers (complete in regard to N, K and P), (*a*) the germination was often below 60 per cent. and (*b*) the seedlings responded favourably to minute quantities of dried blood, pressed yeast or farmyard manure. Aqueous extracts were generally found to be more potent than whole substances. The beneficial effect was lost if the seeds were sown about a month after addition

of the organic substances. This was traced to the activity of micro-organisms.

2. When (*a*) unsteeped seeds were used for germination, (*b*) temperature and light intensity were adequately controlled, and (*c*) mineral supply was augmented by ash constituents of soil and farmyard manure, or dried blood, no significant response to minute quantities of different organic substances could be obtained. Increased concentration and better distribution of dried blood either as such or as aqueous extract could not produce any effect which was appreciably different from that of the minerals alone.

3. No benefit was derived by adding minute quantities of yeast or dried blood, at different stages of growth, to sand-cultured plants raised exclusively on mineral constituents. Similar results were also obtained when the plants were fertilised with a culture solution or when a different mineral medium (laterite) was used as substrate. The potency of minute quantities of different fractions of farmyard manure was studied and in no case could any beneficial effect, distinct from that of the mineral constituents, be obtained.

4. The results show that under controlled conditions and in presence of complete supply of minerals, minute quantities of the organic constituents of dried blood, yeast or farmyard manure have no appreciable effect on the growth of sand-cultured barley. The general evidence would show that, on sand or soil, organic manures produce the desired effect only when applied in bulk, so the mechanism of their beneficial action in the field is to be looked for in other directions.

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