INVESTIGATIONS ON THE RÔLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part VI. Effect of Injecting* Minute Quantities of Certain Forms of Organic Matter on Plant Growth and Reproduction.

By C. R. Harihara Iyer, M.A., G. S. Siddappa, M.A.,
and
V. Subrahmanyan, D.Sc., F.I.C.,
Department of Biochemistry, Indian Institute of Science, Bangalore.

Received November 17, 1934.

It was shown in the previous communication (Siddappa and Subrahmanyan, 1934) that minute quantities of certain forms of organic matter have comparatively little effect on the growth of sand-cultured plants provided with complete supply of minerals. Evidence was also adduced to show that the beneficial effect of such substances and, indeed, organic manures in general, can be seen only when they are applied in bulk. These observations do not, however, preclude the existence of certain active principles, which, under favourable conditions, would serve as auxiliaries to plant growth and reproduction. Such substances are probably either destroyed or otherwise rendered ineffective under field conditions. The results of the previous study would indeed suggest that, when applied to sand or soil, they are decomposed by microorganisms.

Most of the previous workers who adduced evidence in favour of growth-promoting principles in organic extracts had worked with water-cultured plants (Bottomley, 1914, 1915-17, 1919; Mockeridge, 1920, 1924; Clark and Roller, 1927, 1931; Ashby, Bolas and Henderson, 1928; Ashby, 1929; Olsen, 1930 and others) and their observations would suggest that the active substances are (*a*) water soluble, (*b*) comparatively stable in aqueous solution and (*c*) potent even in minute quantities. Water culture is not practicable in field practice, so, if the active substances are to be utilised to increase crop production, they should be supplied to the plant as aqueous solution and in some manner that will avoid or, at any rate, minimise the risk of decomposition by microorganisms. This would naturally preclude their application to the soil. Among the methods of direct application, the technique of injection appeared to be most feasible. That method has been tried by a number of

* This term has been used for want of a more appropriate one. Although hypodermic injection needles were used in the earlier portion of the present study, no mechanical pressure was employed to force the fluids into the plant.

workers either for the supply of nutrients or control of plant disease (Goff, 1897; Bolley, 1903, 1904, 1906; Rumbold, 1915, 1920; Moore and Ruggles, 1915; Rankin, 1917; Elliot, 1917; Bennett, 1927; Scherer, 1927; Thomas and Haas, 1928; Wann, 1929 and others). In addition to ensuring fairly rapid intake, that procedure would also eliminate all possible changes through either chemical reaction with the soil constituents or microbiological activity. The previous observations of Subrahmanyan and Varadachar (1933) had shown that, under favourable conditions, the plant can take up quite considerable quantities of the injected fluid and show marked response to the treatment. In view of the above, a systematic study of the effect of injecting aqueous extracts of different organic substances at various stages of growth was undertaken.

Experimental.

Effect of injecting aqueous extracts of different organic substances into the sunflower plant (*Helianthus annus* Linn.). *Preliminary studies.*—The trials were carried out with Sutton's "Giant Yellow" variety, which was found by previous experience (Varadachar, 1933) to be eminently suitable for the purpose. The seeds were sown in beds which were liberally supplied with farmyard manure. After the plants had grown to a useful height and thickness, they were carefully uprooted and washed free from adhering soil. After taking the necessary biometric measurements and determining the fresh weight in each case, they were transplanted into glazed earthenwere pots made up with acid-washed sand (30 lbs.) and fertilised with superphosphate (30 per cent. P_2O_5; 3·1 g.), potassium nitrate (1·0 g.) and potassium sulphate (1·3 g.). The plants were watered with distilled water. The nutrients passing into drainage were collected in glass or wax-coated metallic containers and poured back into the pots from time to time.

After the plants had established themselves and the new flush just began to appear, the different fluids were injected into them. The equipment (Fig. 1) consisted of a small-sized, rustless hypodermic injection needle (B) which was attached, through a piece of rubber tubing (C), to a reservoir (D) containing the fluid to be injected. The reservoir was a piece of glass tubing (diameter, 1 cm. and length, about 20 cm.) which was joined to a narrower one (diameter, 3 mm.) bent twice at right angles. It was clamped in position and covered with a test tube (F) to minimise evaporation and risk of infection through dust.

Since it would not be possible to force the fluid into the plant in the same manner as in animal experiments, some tests were first carried out with distilled water to determine the conditions under which spontaneous intake would be facilitated. The rubber tube (C) was pinched between two

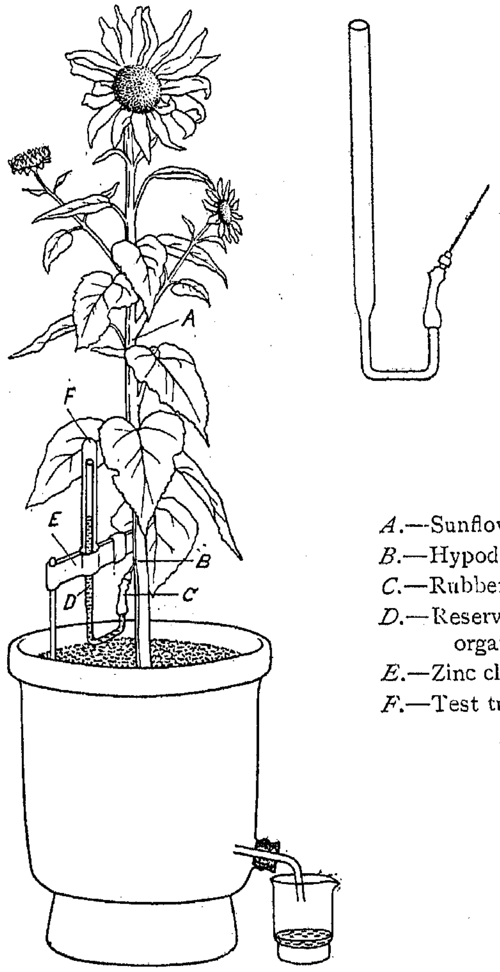

FIG. 1. **Sunflower plant under injection.**

fingers and the reservoir filled to a height of about 15 cm. By gently releasing the fingers, the water was allowed to slowly fill the entire tube and the injection needle, thus displacing all the air from that section of the apparatus. The top of the reservoir was then closed with a finger and the needle (filled with the fluid) carefully introduced into the thickest part of the stem in such a manner that, while retaining a firm hold on the plant, it did not protrude beyond the cambium region.

In the earlier trials, the needle was kept pointing upward as it was felt that it would facilitate rapid movement to the growing parts of the plant. It was found, however, that, with such an arrangement, it was very difficult to keep out air bubbles either before or after introducing the needle. Later trials showed, however, that it was far more convenient to have the needle pointing downward. The equipment for injection was also greatly simplified as will be seen from subsequent studies.

The above test was carried out with each one of the experimental plants. If in any case the water was either not being taken up at all or at a much slower rate than in others, the needle was taken out and then tried in a fresh position. In this manner, it was ensured, even before the commencement of the experiment, that the fluid to be injected would be taken up by all the plants.

It should be admitted, however, that, in spite of these precautions, the intake of water or the extracts which followed did not proceed at the same rate in all the plants. Even when the conditions were identical, some plants took up the fluids at faster rates than others. The difference was sometimes due to partial obstruction near the tip of the needle, but, more often, it was due to some inherent variations between the plants themselves —a condition which could not be adequately controlled. Even the same plant did not take in the fluid at the same rate during the period of observation. The intake was largely influenced by the weather conditions. It was generally observed that bright sunshine, fairly high temperature or low relative humidity led to rapid intake, while wet weather, low temperature or high humidity produced the reverse effect.

After determining the most suitable position for each plant, the needle was fixed in position by bees-wax and the point of entry so covered that none of the injecting fluid could pass out. The major part of the distilled water left in the reservoir was then pipetted out leaving only a small quantity as seal against the entry of air. The plants were then divided into five batches and treated as follows :—(*a*) distilled water (control), (*b*) yeast extract, (*c*) extract of dried blood, (*d*) extract of farmyard manure, and (*e*) effluent from activated sludge. In each case, 10 c.c. of the fluid was introduced into the reservoir and the intake allowed to proceed in the normal way.

The yeast extract used in the present study was prepared by treating dry, powdered brewery yeast (30 g.) with 300 c.c. of cold water and shaking the suspension for 30 minutes. After settling, the clear supernatant was passed through fine filter paper. The filtrate was used for the injection. It contained 2·51 per cent. of solid matter.

The extracts of farmyard manure and dried blood were also prepared in a similar manner. The farmyard manure was a well rotted product taken from the interior of a heap. The extract prepared out of it was red brown in colour and possessed an earthy odour. Its solid content was 0·27 per cent. The dried blood was a commercial product with a total nitrogen content of 10·3 per cent. The extract from it was greenish yellow in colour and possessed a strong, unpleasant odour. Its solid content was 0·79 per cent.

The effluent was obtained from the suspension in the re-aeration tank.

The sludge was allowed to settle and the clear supernatant, which was colourless and odourless, passed through a filter. Its solid content was 0·04 per cent.

After a few days, when useful amounts of the different fluids had passed in, the injections were stopped, the procedure for the withdrawal of the needles being similar to that adopted for introducing them. The quantities taken up by the plants varied from practically all that was added (10 c.c.) to only 2 or 3 c.c. There was no particular preference shown for any fluid, so, those results have not been recorded. The other observations are presented in Tables I and II.

Transplanted on 2nd March 1932 (stage I); distilled water injected on 11th March; extracts injected on 24th March (stage II); biometric measurements taken on 5th April (stage III); plants uprooted and final observations made on 27th April (stage IV).

TABLE I.

Fluid injected	Averages							
	Height in cm.			Girth in cm.			Root length in cm.	
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III	Stage I	Stage IV
Yeast extract	68·6	62·5	67·1	4·7	5·1	5·2	20·3	11·6
Extract of Fy. manure	71·2	64·9	67·7	5·0	5·2	5·3	20·9	13·1
Extract of d. blood	74·0	65·3	66·6	5·1	5·7	5·7	22·3	13·1
A. sludge effluent	69·8	64·1	67·5	5·1	5·7	5·6	22·3	12·1
Distilled water (control)	61·8	59·8	61·9	4·7	5·3	5·2	19·2	9·8

TABLE II.

Particulars	Fluid injected				
	Distilled water (control)	Yeast extract	Extract of dried blood	Extract of Fy. manure	A. S. effluent
Number of plants ..	25	8	12	10	8
Average number of flowers per plant ..	1·8	4·1	2·3	2·8	2·9
Average dry weight of flowers per plant in grams ..	3·7	6·9	2·7	7·6	7·0

It may be noted that, in all the cases, the plants showed some depression shortly after transplanting, but recovered rapidly after that period. The best development was seen in the case of plants injected with sewage effluent which showed significant increase not only in height but also in girth. Those receiving distilled water showed the least progress. The root length was reduced in all the cases partly because of the natural restraint imposed by the pot and partly owing to the nutrients being near the surface of the sand.

Although the plants had originally been raised on manured beds, their subsequent response to injection of minute quantities of different extracts was quite striking. The effect on flowering was most pronounced, not only the number but also the weight showing marked increase in some of the cases. Yeast extract, extract of farmyard manure or activated sludge effluent produced favourable effects, while extract of dried blood led to slight depression.

Experiments with sand-cultured plants.—With a view to studying the effect of injection on plants raised exclusively on mineral media, the sunflower seeds were sown in acid-washed sand (50 lbs.) provided with calcium

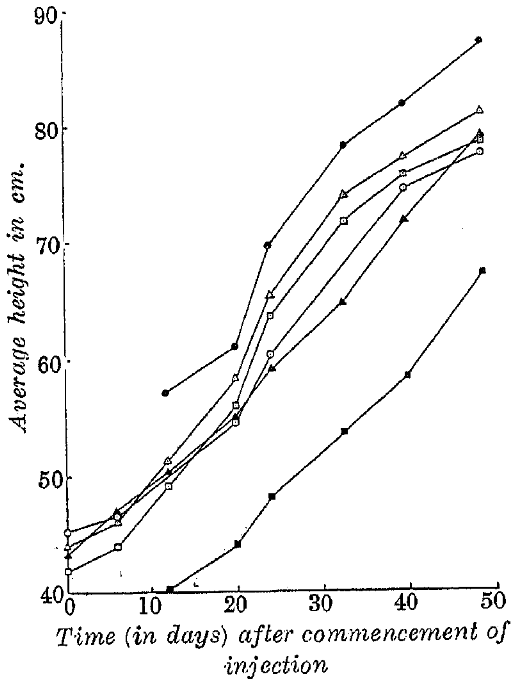

Fig. 2.

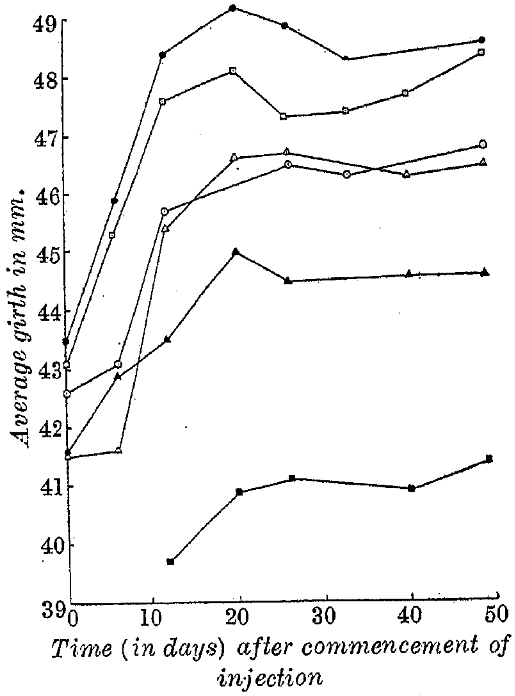

Fig. 3.

Figs. 2 and 3. Effect of injection of organic extracts on the growth of *Helianthus annus.*

●—● Yeast extract
△—△ Soil extract
⊙—⊙ A. sludge effluent
▲—▲ Dried blood
□—□ Farmyard manure
■—■ Untreated (control)

acid phosphate (5·1 g.), potassium nitrate (3·3 g.), and potassium sulphate (2·5 g.). Six seeds were sown per pot and, as the plants came up, four of them were removed at intervals for various biometric measurements. Of the two that were left, one was used for injection while the other was left untreated, as control. Ten pots were allotted for each treatment. The fluids that were injected were the same as in the previous experiment, except that soil extract was also included. The latter was prepared by shaking 30 g. of rich garden soil with 300 c.c. of water and filtering. The other extracts were prepared in the same manner as before. The results have been presented in Figs. 2 and 3 and in Tables III and IV.

Seeds sown on 9th May 1932; distilled water injected on 20th June; organic extracts injected on 23rd June; flower buds emerged on 13th July; watering stopped on 11th August; plants removed and seeds collected on 2nd September.

TABLE III.

Fluid Injected	Before Injection (22-6-1932)				After Injection (11-8-1932)			
	Fresh weight of plant in g.	Dry weight in g.		Root length in cm.	Fresh weight of plant in g.	Dry weight in g.		Root length in cm.
		Stem and leaves	Roots			Stem and leaves	Roots	
Yeast extract	62·9	9·54	1·91	19·4	179·1	23·03	6·57	37·5
Extract of Fy.m.	77·5	12·43	2·83	26·2	155·2	21·46	5·60	38·0
Soil extract	91·4	13·06	3·19	27·0	169·9	18·34	7·08	37·3
A. S. effluent	87·5	13·70	2·66	26·4	152·8	19·67	6·68	36·0
Extract of dried blood	78·6	12·02	2·54	23·6	132·6	17·56	5·42	31·7
Distilled water (control)	57·9	8·70	1·84	22·4	103·4	13·77	4·25	33·4

It may be seen from the results that the plants receiving yeast extract showed the most marked increase not only in height and thickness but also in weight. Farmyard manure came second. Soil extract and sewage effluent came third with more or less similar results. Extract of dried blood was fifth and distilled water (control), last. The observations on flowering and seeding brought out some striking differences between the various treatments. Not only the yields of those two products but also their proportion to the total weight of the plant had been

TABLE IV.

Item	Fluid Injected					
	Yeast extract	Extract of farmyard manure	Soil extract	A. S. effluent	Extract of dried blood	Distilled water (control)
Fresh weight of flower	55·2	48·2	40·9	31·2	25·5	21·2
Dry weight of seed	9·64	8·94	7·03	4·99	4·26	3·29
Dry weight of whole plant	39·24	36·00	32·45	31·34	37·24	25·21
Percentage of dry weight of flower to that of whole plant	24·6	24·8	21·7	15·9	15·6	13·1

greatly altered. The most satisfactory results were obtained in the case of farmyard manure and yeast extract. Soil extract came third. Activated sludge effluent and dried blood extract came very much behind and were only slightly superior to the control.

The above observations, together with those of the previous experiment, would show that, at any rate, some of the organic extracts have profound influence not only on the growth but also in the reproduction of *Helianthus annus*. Attention has already been drawn to the practical significance of some of these findings.* Similar results have also been independently obtained in the case of yeast extract (Virtanen and Hausen, 1933, 1934) by water-culture studies on peas.

The favourable action of some of the extracts may be due to either or both of the following causes: (*a*) they may provide certain substances, which are useful to plant growth and reproduction, (*b*) they may contain some constituents which may act as irritants and force the plants to flower and seed to a greater extent than they might otherwise do. The latter action would, no doubt, be augmented by the injury inflicted by the injection needle.

The existence of growth-promoting substances derives much support from the fact that the plants injected with some of the extracts—especially

* *Soc. Biol. Chemists (India)*, Symposium, July 1932, 8; *Madras Agric. J.*, 1932, **20**, Pt. 11; *Proc. Ind. Sci. Cong.*, 1933, **20**; *Proc. Joint Session, S. Ind. Sci. Assoc., Soc. Biol. Chemists (India)* and *Ind. Chem. Soc.*, April 1933, 11.

those of yeast and farmyard manure—have shown considerable improvement in all directions, as compared with the controls. It is hardly probable, however, that those substances are identical with those responsible for the increased flowering and seeding. A study of the related data would, in fact, show that there is no relation between the effect on vegetative growth and that on reproduction. Furthermore, it may be noted that in the first experiment, there was no marked effect on growth while flowering was favoured by some of the extracts. These observations would show that the two phenomena are distinct and are due to different agencies.

The study of the chemical nature of the active constituents is rendered difficult by the fact that some of the extracts are derived from materials which are themselves mixtures of a number of substances. Moreover, the origin and nature of the starting materials (*e.g.*, farmyard manure and yeast) are so far removed that it is hardly probable that the extracts derived from them contain the same active constituents or such as are even distinctly related to each other. It would appear, therefore, that a number of substances which are not chemically related are responsible for the improved growth and reproduction observed on injection into the plant.

This heterogeneity of character lends support to the theory that the active substances, especially those concerned with flowering and seeding, are not of the nature of plant nutrients but are foreign bodies which act as irritants. All the injected fluids are different in composition and character from those present in plant tissues and it may be naturally expected that they would evoke some physiological response, the effect being more pronounced in some cases than in others. The action of the irritants may explain the increased reproduction, but it is difficult to realize how they could also favour growth. In fact, if such a presumption is granted, the two modes of action outlined above would become identical in practice.

A comparison with the results of the previous study (Siddappa and Subrahmanyan, *loc. cit.*) would show that some of the substances which proved highly potent on injection into the plant were comparatively ineffective when added to sand or laterite. This would support the conclusion that the active substances are destroyed or otherwise rendered ineffective on application to sand or soil. It would also explain why some of the commercial preparations (such as bacterised peat) known to contain substances favouring plant growth, proved comparative failures when tried on the field. The observations would thus show that failing the water-culture (which is not feasible in practical agriculture), the injection method is the most satisfactory way of introducing the active substances into the plant. The conditions must, of course, be further standardised and the procedure greatly simplified before it can be adopted in field practice.

Although the different fluids used for injection contained minute quantities of organic matter, it is yet difficult to state whether the active substances were exclusively carbon compounds. All the extracts contained some mineral matter as well and some of them, especially soil extract and sewage effluent, contained mostly inorganic salts in solution. Further work with purely organic or inorganic substances, as also with mixtures of known composition, is needed before any definite information regarding the chemical nature of the active substances can be obtained.

Injection into tender plants.—The plants that were used for the previous studies were mature ones which generally started flowering shortly after injection. With a view to determining whether similar favourable effects can also be obtained by injection into comparatively tender plants, a series of experiments were carried out during the Winter months of 1932 and Spring of 1933.

The plants were raised on acid-washed sand and minerals, the details being the same as in the previous experiment. The different fluids were injected when the plants were about a month old. The vegetative growth continued for a further period of about six weeks before flower buds began to appear. The usual biometric measurements were taken at intervals and after the seeds had ripened, the plants were removed and the weights determined.

The fluids that were used for injection were the following.—(*a*) yeast extract (20 g. of dry yeast extracted with 400 c.c. of cold, distilled water; total solids, 1·5 g. in 100 c.c.); (*b*) extract of dried blood (prepared in the same manner as *a*; total solids, 0·7 g. in 100 c.c.); (*c*) sterile yeast extract (extraction as in *a*; autoclaved for 15 mins. at 15 lbs.); (*d*) sterile extract of dried blood (prepared in the same manner as *c*); (*e*) potassium nitrate (1 per cent., 10 c.c.); (*f*) neutral potassium phosphate (1 per cent., 10 c.c.); (*g*) KNO_3 (1 per cent., 5 c.c.) + K_2HPO_4 (1 per cent., 5 c.c.); (*h*) dried blood (prepared as in *b*) + K_2HPO_4 (1 per cent., 5 c.c.); (*i*) di-ammonium phosphate (1 per cent., 10 c.c.); (*j*) calcium nitrate (1 per cent., 10 c.c.); (*k*) distilled water, 10 c.c. (control); and (*l*) untreated (control). The object of the trials was to determine whether (*a*) autoclaving destroyed the active constituents, (*b*) any of the inorganic salts can produce effects similar to those of the organic extracts and (*c*) the potency of dried blood can be improved by addition of mineral salts. The experiments were carried out under aseptic conditions (except in those of *a*, *b* and *h*), sterile solutions, needles and tubes being used for the injections. The reservoir tubes were plugged with cotton wool and the point of entry of the needle into the plant protected with wax.

It was found that injection into tender plants was rather difficult, the needles often passing through or the fluids flowing out at the side. In the case of some plants, the injection had to be repeated several times before even a small quantity could be sent in. In spite of these difficulties, nearly a hundred plants were successfully treated with the different fluids.

The biometric measurements, as also the final observations, showed, however, that the response of the plants was quite erratic. Although the average values showed some difference, the deviations were so considerable that no significance could be attached to the results (Table V).

TABLE V.

Fluid injected	Average dry weight in g.			Percentage of weight of seed to that of the whole plant
	Stem and leaf	Root	Seed	
Yeast extract (fresh)	20·69	6·25	6·94	20·4 ± 4·3
Extract of dried blood (fresh) ..	19·36	6·58	7·79	21·9 ± 4·4
Yeast extract (sterile)	21·56	6·33	7·15	20·5 ± 5·1
Extract of dried blood (sterile) ..	19·13	5·84	7·85	24·1 ± 4·6
Potassium nitrate	19·28	6·35	8·39	25·3 ± 9·2
Dipotassium phosphate	19·20	5·08	8·30	27·5 ± 7·8
$KNO_3 + K_2HPO_4$	20·68	4·98	7·55	22·8 ± 5·1
Dried blood + K_2HPO_4	19·64	4·94	8·61	25·0 ± 3·6
Diammonium phosphate ..	17·99	6·33	6·93	21·5 ± 7·2
Calcium nitrate	19·67	5·77	8·70	25·2 ± 5·9
Distilled water (control) ..	21·33	5·49	6·71	20·5 ± 5·9
No injection (control)	16·49	4·94	8·41	26·8 ± 3·6

Attempts to simplify the method of injection.—The procedure followed in the previous experiments was too elaborate and expensive for adoption in field practice. It was considered desirable, therefore, to develop a simpler method of direct feeding which could be followed by even unskilled workers. The use of the injection needle was out of the question, so the puncturing was carried out by either a nail or a small knife as was convenient. The following were some of the methods that were tried.—(*a*) A fairly large hole was bored in the plant and, after introducing the necessary quantity of fluid, the opening was closed with fine clay paste. (*b*) A number of small punctures were made at different points on the stem and a small quantity of fluid introduced into each one of them. (*c*) The procedure was the same as in (*b*), but the fluid was introduced as admixed with soil. (*d*) The treatment was similar to that in (*b*) and the punctures were closed with clay. (*e*) Small bits of bark were removed from different points on the stem and the fluids dabbed on them. (*f*) The barks were just opened with the knife and, after applying the fluid, they were fixed in position and tied round with wax cloth. In addition to sunflower plants, these treatments were also tried on different varieties of crotons, roses, ferns and balsams, as also on lantana, hongay, margosa, sandal and cotton plants.

In the preliminary experiments which were carried out with inorganic salts, it was noted that some plants showed immediate response to the different treatments while others were comparatively slow. Balsam was the most sensitive and lantana the least. Among the various treatments, the first one was not quite favourable. The plants were slow to recover from the shock. Callus formation was tardy and, in some cases, the injury was aggravated by insect attack. The subsequent response was not also significant. The second method was more useful. The wounds healed fairly quickly and the plants showed marked response to the treatments. The third and fourth methods yielded results similar to that of the second one. The fifth treatment was not very helpful. Only small quantities could be introduced by that method. The sixth method, which was more elaborate, also suffered from the same defect as the fifth one. The following results (Table VI) obtained with pot cultured cotton plants treated according to the second method with 0·25 c.c. each of 1 per cent. solutions of a few inorganic salts would be of interest.

There were some interesting features in the response of balsams to the different treatments. Shortly after the injections, practically all the plants showed signs of wilting. The adverse effect was, however,

TABLE VI.

Solution injected	Average No. of flowers per plant	Solution injected	Average No. of flowers per plant
Ammonium hydrogen phosphate	34	Calcium acid phosphate	57
		Magnesium sulphate ..	8
Potassium hydrogen phosphate	76	Sodium bicarbonate ..	27
Calcium sulphate ..	52	Ferric sulphate ..	15

only temporary: in most cases, the plants recovered completely within a few hours. The subsequent observations were varied, depending on the nature of the salt used for injection. Increased flowering was noticed in some cases while profuse leafing was marked in others. When poisonous chemicals such as copper sulphate, potassium cyanide or ferric sulphate was used for injection, the plants never recovered from the initial wilting. In the case of copper sulphate, a dark ring was noticed in the immediate vicinity of the point of injection, showing the extent of spread of the poison. Similar rings were also noticed in the other two cases though the coloration was not so pronounced.

In addition to the foregoing, a number of experiments were carried out introducing the different chemicals, in the solid condition, into punctures made in the manner already described. In some cases, the solids were also applied as admixed with sand or soil. It was noted that, in all the cases, the chemicals were taken up fairly rapidly. The subsequent effects were not, however, quite pronounced, because, in most cases, the plants were slow to recover from the effects of high concentrations of the salts. Even profuse watering did not appreciably improve the condition. In the case of some palms and crotons, favourable effects were noticed about six months after the treatment, while, in others, no perceptible effects could be observed. In view of this and the difficulty in introducing known quantities of solids into the plants, it was considered desirable to use only aqueous solutions or suspensions for subsequent injections.

Injection of inorganic chemicals.—Pot-culture experiments with French beans (*Phaseolus vulgaris*).—With a view to determining the extent to which the minute quantities of inorganic constituents present in the different extracts would affect plant growth and reproduction, an extensive series of trials were carried out applying pure chemicals to pot- as well as plot-cultured plants. For pot experiments with French beans, the soil (30 lbs.) was treated with burnt lime (20 g.) prior to application of farmyard manure (200 g.). The soil was then rested for 10 days after which seeds were sown at the rate of four per pot. After the seedlings came up, they were reduced to two per pot. Twelve plants were allotted for each chemical and eleven salts were tried for each treatment. The following were the treatments.—(A) The chemicals (1 g. each) were applied in one lot prior to sowing. (B) The treatment was similar to that in (A) except that the salts were applied just before flowering. (C) The chemical was divided into two equal lots, one of which (0·5 g. per pot) was applied at the same time as (A) and the other at that of (B). (D) The chemicals were injected into the plants as aqueous solutions or suspensions (1 per cent.). The procedure for injection was very simple and consisted in making five small punctures in the stem of each plant just prior to flowering. One drop (0·05 c.c.) of the solution or suspension was introduced into each puncture so that the total quantity taken up by each plant was 0·25 c.c. and the corresponding quantity of chemical, 2·5 mg. The plants were kept in the open and watered in the usual way. Biometric measurements were made at intervals. When the pods were dry, the plants were uprooted and the dry weights of different parts determined.

It was observed that the average plant heights and girths, as also leaf areas, were more or less similar and yielded no clue to the actual effects of the chemicals in the different cases. The numbers of pods and flowers were misleading. The average weights of pods were also variable. In view of these findings, the data relating to the above have not been recorded. The other results have been presented in Table VII.

It may be seen from the results that, except in a few cases, injection of inorganic chemicals had reduced the total dry weight and depressed the yield of pods.

The quantities used for injections were very small and were only four-hundredths of those applied to the soil. They were, nevertheless, highly potent as may be seen in the case of copper sulphate, which killed out the plant within a few days. The best results were obtained when the chemicals were applied to the soil in two lots—one in the early stages and the other just prior to flowering. Among the various chemicals, the two phosphates (potassium and calcium acid) proved to be the best. They improved not only the yield of pods but also the ratio of pods to the rest of the plant. Although this effect is similar to that observed with

Seeds sown on 16th May 1933; second set of top dressings applied on 19th June; chemicals injected on 20th and 21st June; pods removed and plants uprooted between 24th and 28th July.

TABLE VII.

Chemical used for treatment	Series A			Series B			Series C			Series D		
	Average weight of plant in grams	Average weight of pods per plant in grams	Wt. of pods / Wt. of stem & roots	Average weight of plant in grams	Average weight of pods per plant in grams	Wt. of pods / Wt. of stem & roots	Average weight of plant in grams	Average weight of pods per plant in grams	Wt. of pods / Wt. of stem & roots	Average weight of plant in grams	Average weight of pods per plant in grams	Wt. of pods / Wt. of stem & roots
Calcium sulphate ..	6·48	5·36	4·8	8·13	6·75	4·9	8·25	6·63	4·1	8·61	7·12	4·8
Potassium hydrogen phosphate ..	5·86	4·72	4·1	..	..	..	11·18	9·57	5·9	6·63	5·30	4·0
Copper sulphate ..	6·33	5·09	4·1	..	..	..	7·11	5·62	3·8	Plants died		
Magnesium sulphate ..	7·83	6·33	4·3	..	..	..	9·01	7·55	5·2	5·54	4·67	5·4
Ammonium hydrogen phosphate ..	9·43	7·59	4·1	..	..	..	8·32	6·95	5·1	5·94	4·78	4·1
Ammonium sulphate ..	8·40	6·80	4·2	9·68	8·22	5·6	7·92	6·45	4·4	8·39	7·02	5·1
Sodium bicarbonate ..	7·82	6·22	3·9	..	..	..	7·84	6·23	3·9	7·14	5·99	5·2
Ferric sulphate ..	7·78	6·33	4·4	9·54	7·77	4·4	10·71	8·96	5·1	7·91	6·38	4·2
Calcium hydrogen phosphate	7·93	6·30	3·9	7·18	5·85	4·4	12·93	11·00	5·8	7·23	5·83	4·2
Calcium chloride ..	7·59	6·06	4·0	7·76	6·43	4·8	10·67	8·70	4·4	6·8	5·67	5·0
Manganous sulphate ..	9·03	7·17	3·9	6·46	5·18	4·0	9·37	7·47	3·9	7·75	6·38	4·7

Untreated (control) average wt. of plant, 6·42 g.; Average wt. of pods per plant, 5·16 g.;

$$\frac{\text{Wt. of pods}}{\text{Wt. of stem \& roots}} = 4{\cdot}1$$

.. Experiments spoilt or data insufficient.

some of the organic extracts, the modes of action in the two cases would appear to be different. The organic extracts were comparatively ineffective when applied to the soil (Siddappa and Subrahmanyan, *loc. cit.*), but were potent when injected into the plant. The reverse was found to be the case with the phosphate.

Pot-culture experiments with barley.—The details relating to manuring and sowing were the same as those of the previous series. The treatments were also similar except that (*a*) one of the series (B) in which the chemical was applied to the soil, as a single dose, just prior to flowering was not included, (*b*) a larger number of chemicals were tried and (*c*) in the case of chemicals which were sparingly soluble, the stability of the suspensions used for injection was improved by addition of 0·1 per cent. agar.

As in the case of French beans, the biometric measurements were rather misleading and have not therefore been recorded. It may be stated, however, that the plants receiving the chemicals in two instalments, through the soil (series C) were generally healthier and better developed than the others. The injected plants were mostly poor, those receiving copper, manganese or iron sulphate being the worst affected. The latter did not, in fact, recover from the initial shock and died within a few days after the injection. The final observations have been presented in Table VIII.

It may be seen from the results that the application of chemicals either directly (by injection) or through the soil had generally led to greater production of straw than of grain. The total yield had, no doubt, been increased in many cases, but the ratio of grain to straw was mostly lower than that of the control. This effect is prominently seen in the injection series in which the chemical treatments would appear to have had a marked depressing effect.

Plot experiments with French beans.—On a uniform piece of land, forty-four square plots, each measuring 10′ × 10′, were laid out. The plots were separated from each other by ridges of 1 foot width and 9 inches height. Farmyard manure was applied at the rate of 50 lbs. per plot (approximately 10 tons per acre) and, after resting for 10 days, superphosphate was added to 250 grams per plot (about 2 cwts. per acre). The plots were then sown with French beans, carefully graded seeds being used for the purpose. Sixty-four seeds were sown per plot, each seed being separated from its neighbour by 1 foot. The plants were watered with filtered water, the same quantity being applied to each plot. Care was taken to ensure that the plants came up uniformly well, those which were unsatisfactory being uprooted and replaced by plants from an adjacent area which had been maintained

Seed sown on 14th June 1933; first set of top dressings applied on 28th June; chemicals injected on 18th July; second top dressing given on 21st July; plants harvested on 6th September.

TABLE VIII.

Chemical used for treatment	Series A				Series C				Series D			
	Average yields per plant			$\frac{\text{Grain}}{\text{Straw}} \times 100$	Average yields per plant			$\frac{\text{Grain}}{\text{Straw}} \times 100$	Average yields per plant			$\frac{\text{Grain}}{\text{Straw}} \times 100$
	No. of ears	Weight of grain in g.	Weight of straw in g.		No. of ears	Weight of grain in g.	Weight of straw in g.		No. of ears	Weight of grain in g.	Weight of straw in g.	
Calcium chloride	4·2	2·51	4·08	61·5	5·0	1·85	2·91	63·6	5·5	2·30	3·61	63·7
Calcium sulphate ..	5·0	2·36	3·33	70·9	5·0	2·81	3·09	90·9	4·0	2·32	3·65	63·6
Calcium acid phosphate ..	3·7	1·95	3·23	60·4	4·3	2·68	3·12	85·9	4·3	2·44	3·10	78·7
Potassium hydrogen phosphate	5·2	2·97	3·05	97·4	3·7	3·26	3·60	90·6	5·0	2·41	4·91	49·0
Ammonium hydrogen phosphate	2·5	1·81	3·70	48·9	5·5	3·98	4·25	93·6	5·0	2·16	2·88	78·5
Ammonium sulphate ..	5·5	4·05	3·78	107·1	7·5	2·99	4·41	67·8	3·7	1·83	3·48	52·9
Potassium sulphate ..	4·2	2·26	2·90	77·9	4·2	2·53	4·22	60·0	3·5	2·08	2·55	81·6
Copper sulphate	3·5	2·02	2·72	74·3	4·3	1·51	4·09	36·9	—	Plants	died	—
Magnesium sulphate ..	3·7	1·96	2·39	82·0	3·8	2·20	3·28	67·1	5·2	2·57	3·77	68·2
Manganous sulphate ..	4·5	1·80	2·90	62·1	4·8	2·19	3·34	65·6	—	Plants	died	—
Ferric sulphate	4·8	2·03	2·95	69·9	2·8	1·49	2·43	61·3	—	Plants	died	—
Sodium nitrate	5·3	2·45	3·13	78·3	4·3	2·93	3·38	86·7	4·3	1·30	3·28	39·6
Sodium bicarbonate ..	4·0	2·28	2·60	87·7	4·2	1·84	3·02	60·9	4·1	1·70	2·71	62·7
Sodium chloride	3·7	2·39	3·06	78·1	3·8	2·73	2·92	93·5	3·3	1·61	2·96	54·4

Untreated (control) No. of ears per plant, 4·3; Wt. of grains in g., 2·55; Wt. of straw in g., 2·70; $\frac{\text{Grain}}{\text{Straw}} \times 100 = 94·4$.

under the same conditions as the experimental plots. When the plants were on the point of flowering, they were injected with various chemicals, the technique being the same as that adopted in the pot experiments. Four plots, chosen at random, were allotted for each treatment. In addition to the usual biometric measurements on standing crop, representative specimens of plants (four for each plot) were removed at short intervals and the average root lengths and dry weights of different parts determined. After the seeds had set and the pods were dry, the remaining plants were removed and the yields estimated.

As may be naturally expected, the plot-cultured plants were generally healthier and better developed than those previously raised in pots. The periodic biometric measurements did not bring out any prominent difference between the various treatments. The following general observations may, however, be of some interest. The average plant height increased upto a point (about 30 cm.) and then diminished (to about 25 cm.) within the last three weeks of observation. This was presumably due to the weight of the

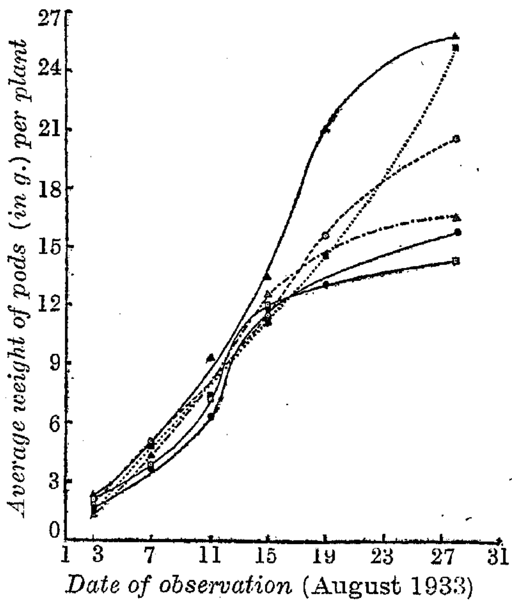

FIG. 4.

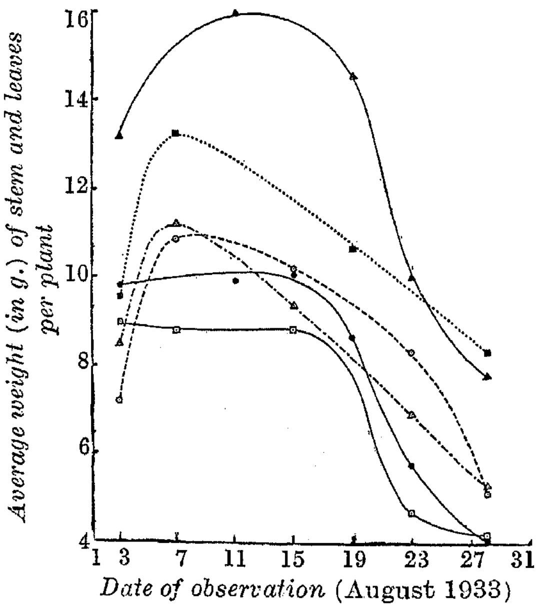

FIG. 5.

Figs. 4 and 5. Response of French beans to injection of different inorganic chemicals.

●——● Ammonium sulphate
○- - -○ Potassium sulphate
■·····■ Potassium nitrate
▲——▲ Calcium acid phosphate
□——□ Distilled water
△-·-△ Untreated (control)

pods which were then forming in large numbers. The roots also showed marked diminution in length (from about 15 to just over 10 cm.) shortly before harvest. They tended, however, to become heavier and more bushy, so that it would appear that the liberal watering combined with easy access to the nutrients made the plants shallow rooted. The other important observations have been recorded in Figs. 4 and 5 and in Table IX.

Farmyard manure applied on 10th June 1933 and superphosphate on 20th June; seeds sown on 22nd June; chemicals injected between 19th and 22nd July; samples taken on 19th, 25th, 28th and 31st July and on 3rd, 7th, 11th, 15th, 19th and 23rd August; plots harvested between 28th August and 2nd September.

TABLE IX.

Observations at the time of harvest.

Chemical injected	Average per plant			Total yield (from 256 plants)		Wt. of pods / Wt. of stem, leaves and roots
	Height in cm.	Root length in cm.	Number of pods	Dry weight of whole plants in Kg.	Dry weight of pods in Kg.	
Ammonium sulphate	26·0	7·9	12·4	5·13	4·08	3·9
Calcium sulphate	28·3	7·8	16·8	6·85	5·36	3·6
Calcium chloride	26·4	8·4	13·9	6·28	4·75	3·1
Tricalcium phosphate	26·0	9·0	13·4	5.08	3·92	3·4
Calcium acid phosphate	27·6	9·5	18·1	8·48	6·59	3·5
Potassium hydrogen phosphate	25·9	9·4	16·5	7·65	6·07	3·8
Potassium sulphate	25·8	9·1	15·0	6·59	5·27	4·0
Potassium nitrate	29·1	10·1	16·8	8·59	6·46	3·0
Potassium chloride	26·8	7·5	13·4	5·66	4·37	3·4
Distilled water (control)	25·3	8·6	11·5	4·73	3·66	3·4
Untreated (control)	26·3	7·8	14·0	5·58	4·22	3·1

It may be seen from Fig. 4 that, in all cases, the dry weight of pods increased steadily until it attained the maximum value at the time of harvest. On the other hand, the dry weight of stem and leaves (Fig. 5) either increased or remained more or less stationary for about two weeks and then diminished rapidly, finally reaching the lowest value at the time of harvest. These observations would show that, in the early stages, the products of assimilation moved directly to the pods without any appreciable intermediary storage in stem and leaves. When the pods began to mature, there was further draft of solids into that region, not only fresh products but also a portion of the materials already stored in stem and leaves being thus translocated. These observations are generally in accordance with those of Narasimhacharya and Sastri (1931) on the tissue fluids of *Phaseolus vulgaris*.

The movement of solids into the pods proceeded at more or less similar rates in all the cases (Table X). It does not appear to have been influenced by the nature of the chemical injected into the plant. The injury inflicted prior to the introduction of the chemical did not make any appreciable difference to the ratio of pods to the whole plant, the figures being about the same for the experimental as well as the control (untreated) plants.

TABLE X.

Chemical injected	Percentage of pods to the whole plant — Number of days after injection						
	14	18	22	26	30	34	39
Ammonium sulphate	13·4	27·9	38·8	54·1	60·2	66·3	79·6
Calcium sulphate	12·3	25·5	32·7	49·8	59·3	69·1	78·3
Calcium chloride	16·7	29·9	38·3	52·6	59·2	66·6	75·6
Tricalcium phosphate	14·2	29·4	34·9	54·2	60·2	67·8	77·1
Calcium acid phosphate	13·4	26·5	36·7	51·8	59·1	68·3	77·8
Potassium hydrogen phosphate	15·0	27·9	34·6	51·8	57·3	66·7	79·3
Potassium sulphate	17·2	31·6	40·3	53·2	60·1	66·5	80·0
Potassium nitrate	13·6	27·1	39·7	55·6	57·8	69·5	75·2
Potassium chloride	15·3	29·5	44·9	54·6	62·6	67·7	77·2
Distilled water (control)	15·2	29·7	42·9	54·6	61·1	65·4	77·5
Untreated (control)	12·7	28·1	41·4	57·3	60·3	68·2	75·7

It would thus be seen that the mode of action of organic extracts (such as that from yeast) is different from that of inorganic salts. Some of the latter, *e.g.*, potassium nitrate or calcium acid phosphate, do, no doubt, facilitate increased assimilation, but they have no influence on subsequent distribution within the plant.

When considering the efficiency of different treatments, some allowance should be made for the nature of the crop. French beans are naturally highly efficient (to the extent of about 80 per cent.) in transforming the products of assimilation into seeds. It would be difficult to improve on such a process or, at any rate, produce a better effect that can be easily realised in field practice. In view of this, it is considered desirable to carry out trials with other agricultural crops, so as to further distinguish between the effects of organic extracts and inorganic salts.

Experiments with horticultural plants.—Through the courtesy of the Superintendent to Government Botanic Gardens in Mysore, some experiments were conducted in Lal-Bagh, Bangalore, injecting different organic extracts, as also inorganic salts, into balsams, zinnias, cosmos and a few other flowering plants. The pots were prepared with red earth and leaf mould as is usually done in horticultural practice. Liberal watering was also given. When the plants had grown to useful heights and thickness, the different fluids were injected, a simple modification of the original procedure being adopted for the purpose. A short piece (length about 10 cm.) of glass tubing (internal diameter 1 cm.) was drawn to a narrow point, the end being sufficiently sharp to enter the plant without much difficulty. The lower part of the tube was bent slightly to one side, so that, while the injection was in progress, the tube (which would also constitute the reservoir) might remain parallel to the plant. To commence the injection, the fluid was sucked into the tube and after closing the top with the thumb, the sharp end was introduced into the plant. The tube was then tied to the stem of the plant and the top covered with cotton wool to prevent the entry of dust.

It was observed that in addition to being very convenient, the new procedure was even more efficient than the elaborate equipment with the hypodermic injection needle. The fluids were taken up very rapidly, 4–5 c.c. being the average per plant for a day. In some cases, the entire contents of the reservoir (about 10 c.c.) were taken up on standing overnight.

The response of the plants was not, however, quite satisfactory. In some cases, striking changes were noticed, while in others receiving the same treatment, none could be observed. There was considerable variation among the plants themselves, probably owing to some inherent difference

TABLE XI.

Solution injected	Average dry weight (in mg.) of seed per plant	Solution injected	Average dry weight (in mg.) of seed per plant
Sodium hydrogen phosphate	50	Yeast extract ..	134
Potassium chloride ..	47	Extract of dried blood	109
Manganous sulphate ..	50	Extract of farmyard manure	154
Ferric sulphate ..	Plants died	Soil extract	123
Potassium nitrate ..	140	Distilled water (control)	88
		Untreated (control) ..	81

among the seeds used for sowing. In addition to this, some of the experimental plants were disturbed by monkeys which were present in the neighbourhood. In view of these defects, the data relating to yields have not been recorded. The above observations (Table XI) relating to balsams (variety, Prince Bismarck) may, however, be of some interest.

Discussion.

The present enquiry has brought to light a number of interesting features in regard to the rôle of organic matter in plant nutrition. Some of the findings also hold out possibilities of practical application.

The injection experiments with *Helianthus annus* have shown that, under certain conditions, not only the general growth, but also the ratio of flower and seed to the rest of the plant can be greatly improved by direct feeding of organic extracts. In addition to supporting the findings of previous workers in regard to the existence of substances which are helpful to plant growth, these observations also show that the extracts contain certain constituents which exercise marked influence on plant reproduction. The latter observation is of much practical significance, because, in the case of most agricultural and horticultural crops, the value of the produce depends on the yield of flowers, fruits or seeds, the increased output of which—even without any change in the total weight of the plant—would mean bigger returns to the producer.

The foregoing observations are in agreement with those of Virtanen and Hausen (*loc. cit.*) on water-cultured peas. The two sets of results obtained, by separate methods and independently of each other, would show conclusively that the active constituents are water soluble and can be either taken up through the roots or introduced directly by injection.

The previous research (Siddappa and Subrahmanyan, *loc. cit.*) has clearly shown the futility of applying minute quantities of different organic substances to soil or sand cultured plants provided with complete supply of minerals. The active substances are destroyed on standing for some time, so that, if they are to be utilised in field practice, they should be either (*a*) supplied in such large quantities or so frequently that some will always remain in the soil and thus be available to the growing plant, or (*b*) fed directly to the plant in some manner that will ensure rapid intake and will, at the same time, eliminate the danger of decomposition in the soil. The evidence, so far available, is not sufficient to decide as to which of the above will prove most satisfactory in field practice. Further systematic research, both in the laboratory and on the field, will be needed to settle that point.

Some of the earlier observations on *H. annus* suggested that the constituents favouring growth were different from those responsible for increased flowering and seeding. This inference received support from the response of barley and French beans to pure, inorganic salts. Some of the latter were, no doubt, helpful, but their mode of action was different from that of the organic extracts. The minerals facilitated increased assimilation, but did not appreciably alter the distribution of matter between the different parts of the plant. If it be assumed that the minerals present in the extracts behaved in a manner similar to that of the pure substances, it would then follow that the organic constituents were primarily responsible for the increased flowering and seeding.

The mechanism of the action of the different extracts is still obscure. The available evidence is not sufficient to show whether they provide certain nutrients not ordinarily found in the soil or merely function as plant irritants. The conditions relating to the application of the active substances so as to obtain the best results under field conditions have yet to be standardised. Application to the soil may prove largely wasteful, while methods of direct feeding are too elaborate for extended adoption in agricultural practice. Further work on these and allied problems is in progress and will form the subjects of later communications.

Summary.

(1) Injection of minute quantities of certain organic extracts into mature sunflower plants led to not only better growth but also greatly increased flowering and seeding. The best results were obtained in the case of plants receiving extract of yeast or farmyard manure: the total yield of flower and seed was nearly tripled and the ratio of seed to the rest of the plant nearly doubled as compared with the untreated (control) plants. Dried blood was comparatively ineffective. Injection into tender plants did not lead to any significant improvement in yield.

(2) Comparative trials with inorganic salts which were fed directly to pot- or plot-cultured French beans or barley did not lead to any marked improvement, more satisfactory results being obtained by applying the same salts (though in larger quantities) to the soil. In the latter case, the beneficial effects could be traced to increased assimilation and better general development rather than to any alteration in the ratio of seed (pod or grain) to the rest of the plant.

(3) The practical significance of the above and other observations has been discussed.

In conclusion, the authors wish to express their appreciation of the valuable assistance rendered by Mr. K. Saptha Rishi during the short period of his association with the work.

REFERENCES.

Ashby .. .. .. *Ann. Bot.*, 1929, **43**, 333, 805.

Ashby, Bolas and Henderson .. *Ann. Bot.*, 1928, **42**, 771.

Bennett .. .. .. *Calif. Exptl. Sta. Rept.*, 1927, 85.

Bolley .. .. .. *N. Dakota Exptl. Sta. Ann. Rept.*, 1903, **14**, 55; *ibid.*, 1904, **15**, 56 ; 1906, **17**, 35.

Bottomley .. .. .. *Proc. Roy. Soc.*, 1914, **B 88**, 237; 1915-17, **B 89**, 481 ; *ibid.*, 1919, **B 91**, 83.

Do. .. .. .. *Ann. Bot.*, 1914, **28**, 531 ; 1920, **34**, 345.

Clark and Roller .. .. *Soil Sci.*, 1924, **17**, 193; 1931, **31**, 299.

Elliot .. .. .. *Phytopath.*, 1927, **17**, 51.

Goff .. .. .. *Wisc. Agric. Exptl. Sta. Ann. Rept.*, 1897, **14**, 272.

Mockeridge .. .. .. *Biochem. J.*, 1920, **14**, 432.

Do. .. .. .. *Ann. Bot.*, 1924, **38**, 723.

Moore and Ruggles .. .. *Science*, 1915, **42**, 33.

Narasimhacharya and Sastri .. *J. Indian Inst. Sci.*, 1931, **14 A**, 1.

Olsen .. .. .. *Compt. Rend. Trav. Lab. Carlsberg*, 1930, **18**, 1.

Rumbold .. .. .. *Phytopath.*, 1915, **5**, 225.

Do. .. .. .. *Amer. J. Bot.*, 1920, **7**, 1.

Scherer .. .. .. *Phytopath.*, 1927, **17**, 51.

Siddappa and Subrahmanyan .. *Proc. Ind. Acad. Sci.*, 1934, **1**, 229.

Subrahmanyan and Varadachar.. *Curr. Sci.*, 1933, **1**, 343.

Thomas and Haas .. .. *Bot. Gaz.*, 1928, **86**, 355.

Varadachar .. .. *J. Indian Inst. Sci.*, 1933, **16 A**, 129.

Virtanen and Hausen .. *Nature*, 1933, **132**, 408 ; 1934, **133**, 383.

Wann .. .. .. *Amer. J. Bot.*, 1929, **16**, 844.

2123-34.—Printed at The Bangalore Press, Mysore Road, Bangalore City, by T. Subramania Aiyar, Superintendent, and Published by The Indian Academy of Sciences, Indian Institute of Science, Bangalore.