THE USE OF AJOWAN [1] AND MOHUA [2] CAKES AS FERTILISERS.

Through the kindness of the Wardle Engineering Co., Hyderabad (Deccan), samples were obtained of *ajowan* and *mohua* cakes which are industrial waste-products in many districts. These have been examined for their value as fertilisers.

Part I.—Ajowan Cake.

The ajowan cake was not completely free from thymol, the smell of which was quite perceptible, but attempts to extract this by steam distillation showed that the quantity was extremely small. As, however, even this insignificant quantity might have a deleterious effect on bacterial growth and activity, it was considered necessary to investigate this point. For this purpose, two tubes of sterile water inoculated with 1 gram of soil and to one of which 1 gram of the powdered cake was added, were kept at ordinary temperature for two days, by which time a luxuriant bacterial growth was evident in both. A count of the organisms present, made by plating out on synthetic agar, gave the following results :—

Soil control	....	2900 colonies per gram.
Soil + ajowan cake	....	3800 ,,

The results conclusively prove that the amount of thymol present in the cake was quite insufficient to check bacterial activity in the soil.

Analysis of the cake for nitrogen, potash and phosphoric acid, the three chief ingredients of manurial value, gave the following results :—

Nitrogen	....	0·862	per cent.
P_2O_5	....	0·856	,,
K_2O	....	2·02	,,
Ash	....	12·7	,,

Nitrogen was determined by the Kjeldhal method, the phosphate estimated as Mg-pyrophosphate and potash by the perchloric acid method.

These results show that the cake is of only moderate manurial value as it contains comparatively little nitrogen and phosphoric acid

[1] Ajowan, *Carum copticum*, the seed of which yields about 1 per cent. of thymol.
[2] Mohua, *Bassia longifolia*.

though potash is present in appreciable amount. The material might, however, be of use if the nitrogen could readily be made available. It was considered that this point might be tested by the use of 'activated sludge,' the latter containing all bacteria necessary for the purpose.

Ten grams of ajowan powder were accordingly thoroughly mixed in a mortar with 100 c.c. 'activated sludge' and the total volume made up to 300 c.c.; 100 c.c. were taken for immediate analysis while 100 c.c. were aerated continuously for six days. At the end of this period the experiment was stopped, the samples filtered and the nitrogen determined.

TABLE I.

		Control. Ajowan powder + activated sludge 5-7-1923.		Aeration stopped after six days 10-7-1923.	
Nitrogen (free ammonia)	...	0·84	mgms.	4·2	mgms.
,, (albuminoid ammonia)	...	6·0	,,	3·36	,,
,, (nitrate and nitrite)	...	1·0	,,	1·44	,,
Total nitrogen in extract	...	21·84	,,	26·88	,,
,, in residue	...	97·94	,,	99·87	,,
Grand total ...	...	119·78	,,	126·75	,,
Active insoluble nitrogen	...	19·8 per cent.		26·45 per cent.	
Dry weight of the residue	...	3·0756 mgms.		2·5704 mgms.	
Percentage loss of dry weight	...			16·43	

These results lead us to conclude that in spite of its low nitrogen-content the cake might be of use, much of the protein nitrogen appearing to be in a labile form. Experiments on the nitrification value of this cake in soil confirm the above results.

In view of the fact that the total nitrogen in the experiment which was stirred for six days exceeded that in the control, it was thought that the cake might assist nitrogen-fixation. To test this, flasks containing 20 grams of sand (washed with hydrochloric acid), 1 gram of $CaCO_3$, 5 grams of ajowan powder and 100 c.c. of water were sterilised in the autoclave. In another series $CaCO_3$ was replaced by $MgCO_3$ in view of the statements made by some workers that the latter is more efficient than $CaCO_3$ in helping nitrogen-fixation. The flasks were all inoculated with 1 c.c. pea soil. The control-pots were then again sterilised and all were incubated for six weeks, after which period the mixtures were analysed for total nitrogen by the Gunning method. The results are given below :—

		Gain or loss
i. Control	101·09 mgms. nitrogen.	
ii. With $CaCO_3$	102·76 ,, ,,	1·75 mgms. nitrogen (gain)
iii. With $MgCO_3$	86·2 ,, ,,	14·89 ,, ,, (loss)

While with $CaCO_3$ there was practically no change in the nitrogen-content, $MgCO_3$ involved a distinct loss amounting to 14·89 mgms. There is therefore no experimental evidence to support the view that the ajowan cake stimulates nitrogen-fixation.

As only small quantities of the cake were available no further experiments such as pot or plot-cultures could be made; but the experiments mentioned above clearly prove that the cake does not diminish the activity of soil micro-organisms, while its nitrogen appears to be in a form readily available to the plant, at any rate after adequate treatment. In districts where it is obtainable at a low cost it might, therefore, be used as a fertiliser.

Part II.—Mohua Cake.

Analysis of mohua cake gave the following results :—

Oil	....	8·06	per cent.
Nitrogen	....	2·72	,,
P_2O_5	....	0·12	,,
K_2O	....	2·03	,,
Ash	....	7·56	,,

It will be seen that the cake obtained on pressing the mohua seeds retains a large quantity of oil. It is also moderately rich in nitrogen and might therefore be expected to give useful results as a manure. Hutchinson,[1] however, has shown that on adding this cake to the soil there is no evidence of nitrification even after several weeks. The nitrogen of the cake must therefore either be present in a very resistant form, or the cake contains some substance which inhibits the action of nitrifying bacteria. Treatment of the cake with 'activated sludge' was therefore carried out in order to ascertain how readily the protein matter could be rendered available.

Experiment I.—10 gms. of mohua cake were thoroughly mixed with 100 c.c. 'activated sludge' in a mortar and the volume made up to 300 c.c.; 100 c.c. were taken for aeration while another 100 c.c. were used for preliminary analysis. During aeration, however, great difficulty was experienced from frothing as even with a slow current of air this took place and the material was liable to be carried over from the experimental vessel. The experiments were however continued for six days, at the end of which period the mixture was filtered and analysed as usual. The analyses are given in Table II.

[1] *Agri. Res. Inst. Pusa Sci. Reports*, 1920-21, 28.

TABLE II.

		Mohua cake + activated sludge (control)		Mohua cake + activated sludge. Aeration continued for six days	
Nitrogen (free ammonia)	... ...	0·384	mgms.	0·24	mgms.
,, (albuminoid ammonia)	... ...	1·44	,,	1·55	,,
,, (nitrite and nitrate)	... ...	0·10	,,	0·24	,,
Total nitrogen in extract	... ...	7·14	,,	5·46	,,
,, in residue	... ...	123·30	,,	124·70	,,
Grand total nitrogen ...	... ...	130·44	,,	130·16	,,
Loss in weight per cent.	... ...	4·496			

The figures show that no decomposition of the protein of the mohua cake has occurred. It seemed possible that this might be due, partly at any rate, to the high oil-content of the material. The oil was, therefore, extracted by petroleum-ether in a Soxhlet apparatus and the oil-free cake mixed as before with activated sludge and aerated. Frothing still occurred and was even more marked than before, making it impossible to continue the experiment. The oil is, therefore, not responsible for this phenomenon which is, in fact, due to a saponin.

Experiment II.—Attempts were made to extract the saponin and examine its effect on nitrification. The following method[1] was used.

The finely powdered material was extracted with petroleum ether to remove oil. The residue was then treated with boiling alcohol (60 per cent.), the extract filtered, the alcohol distilled off and the residue evaporated on the water-bath with calcined magnesia. The resulting paste was extracted with boiling alcohol (80 per cent.), filtered and concentrated by distillation. Ether failed to remove anything from the residue which on evaporation gave an amorphous, bitter and very hygroscopic powder, forming an acid solution which frothed greatly and gave the following reactions :—

(1) A flesh coloration with aqueous sodium nitroprusside.

(2) A yellow colour with solid diphenylamine.

(3) A dark purple colour with concentrated sulphuric acid, forming gradually.

(4) Reduction only on continued boiling with Fehling's solution. After hydrolysis a heavy precipitate of CuO was obtained.

(5) The solid material when dissolved in sulphuric acid (3 per cent.) and hydrolysed in the autoclave at 105°C during one hour gave a

[1] *Compt. rend.*, 1912, 155, 844.

heavy precipitate of sapogenin and a solution which reduced Fehling's solution.

(6) Among the sugars in the solution after hydrolysis, glucose and xylose were identified by their osazones. Maltose and arabinose may also be present.

These reactions indicate the compound to be a saponin belonging to the acid group.

To see whether saponin had any effect on nitrification, the following solutions were prepared :—

(I)	Ashby's medium with $CaCO_3$	....	Control I.
(II)	,,	....	With 4 c.c. of 1 per cent. saponin per 100 c.c. solution.
(III)	,, without $CaCO_3$	....	Do.
(IV)	,, ,,	....	Without saponin ; Control II.

100 c.c. of medium were used in each case with 1 per cent. $CaCO_3$ where necessary, and duplicates were run. The flasks were inoculated with equal quantities of a good nitrifying soil and were incubated for a week, after which period the nitrogen present as nitrite and nitrate was estimated in accordance with the standard methods advocated for water analysis (A.O.A.C.). The results are given in Table III. They are calculated on 1 c.c. of the liquid.

TABLE III.

Series		I	II	III	IV
Nitrite nitrogen (mgm.)	....	0·009	Trace.	0·001	0·01
Nitric nitrogen ,,	....	0·3	0·027	0·04	0·2

It will be seen that in series II and III where saponin was added there has been but little formation of nitrate.

The total nitrogen was also determined in each series with the following results :—

Series I	....	13·46	mgms. nitrogen.
,, II	....	13·44	,, ,,
,, III	....	5·53	,, ,,
,, IV	....	11·2	,, ,,

It will be noted that there has been a marked loss of nitrogen in series III which contained saponin but no calcium carbonate, probably due to denitrification being set up.

To confirm the inference that the saponin is responsible for the low availability of the cake the following experiment was arranged :—

(1) 100 gms. of soil + 3·5 gms. raw mohua cake.
(2) ,, fat-free mohua cake.
(3) ,, saponin-free mohua cake.

The moisture was adjusted at 60 per cent. saturation and the experiments were continued in duplicate for three months. At the end of this period nitrate was determined by the phenoldisulphonic acid method. The results are given below :—

Nitric Nitrogen.

Control	....	0·37 mgms. per 100 grms. of soil.
Raw mohua cake	....	0·224 ,, ,,
Fat-free mohua cake	....	0·32 ,, ,,
Saponin-free mohua cake.		1·92 ,, ,,

These experiments clearly prove that the low availability of the cake is due to presence of saponin which inhibits the formation of nitrates. In these circumstances the use of the cake as a fertiliser is hardly practicable.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 18-9-25.]