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EFFECT OF NITROGENOUS BIOFERTILIZERS FOR LEGUMINOUS PLANTS ON NODULATION AND FRUITING

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In the present paper, the importance of biofertilizers has been stressed. It provides information on the preparation of nitrogenous biofertilizers for leguminous plants. Experiments were conducted on Gram and Cowpea, using biofertilizers prepared in P.C.S.I.R. Laboratories. The results have been found encouraging. In addition to 10-15 days early flowering in both the crops, seeds treated with biofertilizer produced healthier and taller plants, with greener leaves and increased yield as compared to those of control.

INTRODUCTION

Legumes are next in importance to cereals as a source of human nutrition. Their unique ability to enrich the soil was clearly appreciated by Greeks and Romans, who developed crop rotation in which legumes figures prominently [1]. Legumes are different from other plants, specially in the sense that they could utilize atmospheric nitrogen through nodular bacteria. The first recorded experimental work appears to be the tests conducted arround 1806 by Saussure [2].

In nature the leguminous plants have nodules developed on their roots inside which the nitrogen fixing bacteria (*Rhizobium* sp.) are symbiotically lodged. These bacteria fix atmospheric nitrogen which is used by the crop plants, and thus, the bacteria act in place of nitrogen fertilizers.

A legume plant having sufficient nodulation is not only able to meet its own nitrogen requirements but can also increase soil's nitrogen contents. If sufficient nodules are formed on the roots of leguminous plants, the resulting symbiotic fixation of nitrogen satisfies the demand for nitrogenous fertilizer.

To achieve this objects, seeds are dressed by the biofertilizers preferably just before sowing. By doing so, maximum number of nitrogen fixing nodules could be produced on the tender roots of the seedlings and would enable the perspective legume plants to use nitrogen from air in greater quantities. The whole process is illustrated in Fig. 1. This method increases the yield by at least 40-50 % without the addition of chemical fertilizer. The use of nitrogenous biofertilizer does not aggravate the menace of salinity as could be done by the constant use of chemical fertilizers for prolonged periods. Nitrogenous biofertilizer improves the physical structure and organic content of the soil; it also provides better water retention capacity.

Our experiments on nitrogenous biofertilizers have been conducted on two important crops, namely; cowpea (*Vigna sinensis*) and gram (*Cicer arietin*). Results of these experiments are reported in the present paper.

MATERIALS AND METHODS

Procurement and selection of nitrogen fixing *Rhizobium* species was made separately from gram and cowpea nodules occuring in nature. Isolation of *Rhizobium* from root nodules was done according to Suba Rao's method [3].

Desired Rhizobia were isolated and cultures were initially maintained on mannitol-yeast extract agar [4]. After an incubation of about 48 hours at 30°C, the liquid medium containing the desired *Rhizobium* sp. was mixed with a reasonable quantity of powdered and dried ordinary charcoal at room temperature. Like gram, cowpea seeds were dressed with the specific biofertilizer (Fig. 2). About

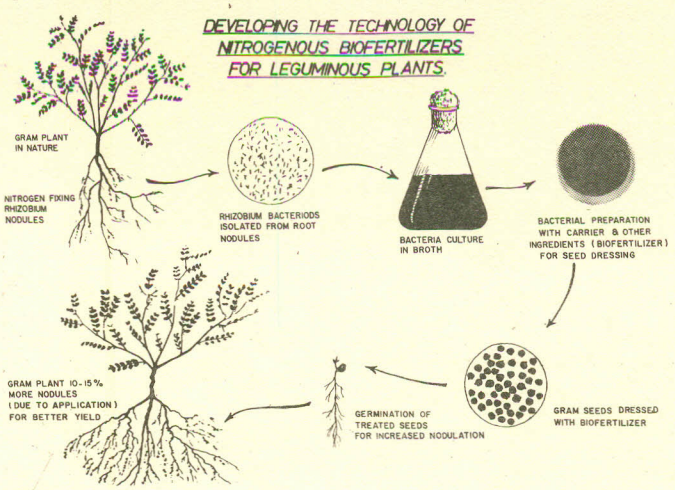

Fig. 1. Diagramatic representation of different stages in the preparation of nitrogenous biofertilizer.

150 g of biofertilizer was made into a paste to treat about 1 kg of seeds. To achieve good results the seeds were dressed with the biofertilizer just before sowing.

Using this nitrogenous biofertilizers, following experiments were conducted in pots, in the Botanical Experimental Plot, as well as P.C.S.I.R. Model Farm at Malir. The results obtained have been presented in Tables 1, 2 and 3.

RESULTS

Experiment No. 1. Four replicates of plastic pots of 8" diameter and 10" depth were filled with finely sieved and sterilized sweet earth. Three sets of replicates were used for testing the three different ratios of biofertilizers. These were in the proportion of 50 g carrier mixed with 50 ml of bacterial culture (1:1), 50 g carrier with 100 ml of bacterial culture (1:2), and 50 g carrier mixed with 150 ml of bacterial culture (1:3). The fourth set was kept as control. Five seeds were sown in each of the pots under experiment in the month of November. The plants were provided with 20 ml of Hoagland nutrient solution [5] on alternate days. This solution does not contain any nitrogen element. After 45 days of growth, the plants were taken out for necessary observations, compiled in Table 1.

After 45 days it was observed that the number and production of bacterial nodules was maximum in the ratio 1:3. The plants were more sturdy, healthy and green as compared to that of control. The control plants showed very poor and retarded growth; the stem and leaves were pale and thin. The presence of greater number of nitrogen fixing nodules is considered to be responsible for healthy growth of plants and improved yield and quality [6].

Experiment No. 2. A second set of control and treated seeds of gram (*Cicer arictimum*) dressed with nitrogenous biofertilizer were sown in the month of December in P.C.S.I.R. Botanical Experimental Field. Two plots of 20' x 20' each for control and treated seeds were prepared using sweet earth. Random samples of ten plants each from control as well as treated with biofertilizer were taken out

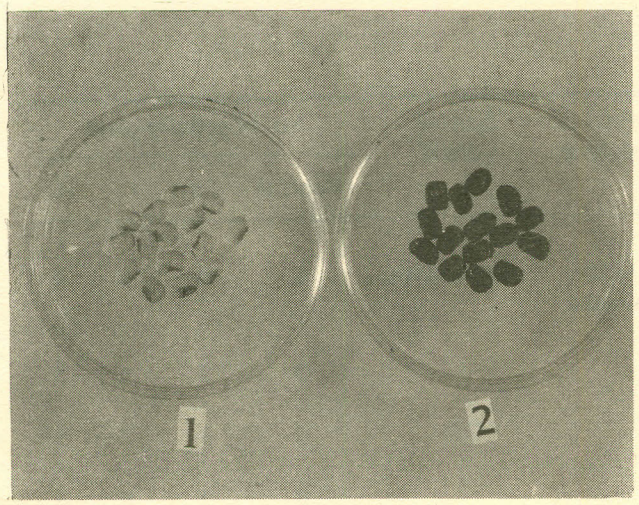

Fig. 2. Cowpea seeds: Showing (1). Seeds without biofertilizer treatment. (2). Seeds treated with biofertilizer.

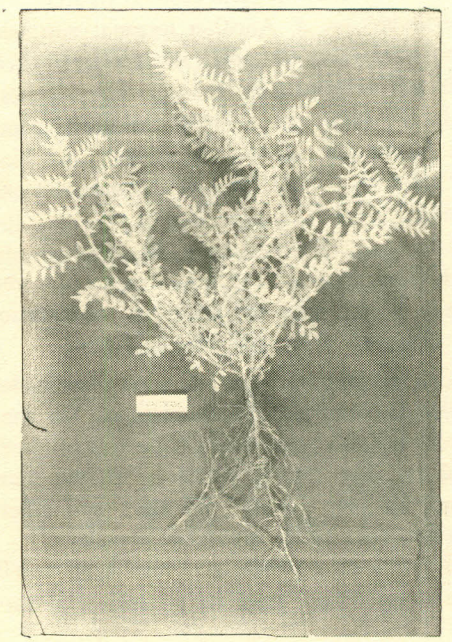

Fig. 3. Gram Plant: Control plant showing smaller number of nodules on the roots.

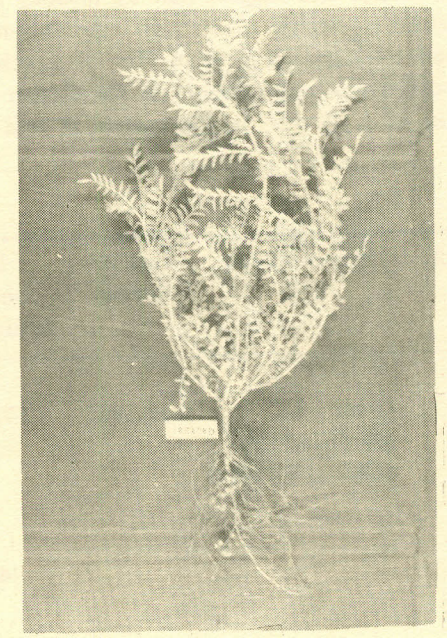

Fig. 4. Treated gram plant showing larger number of nodules on the roots.

after 60 days and observations made. In addition to 10-15 days early flowering the plants in the treated plots were having more nodulation (Fig. 3 & 4), and seemed comparatively greener, healthier, taller and more fruity. Details of the experiments are presented in Table 2.

The above data shows approximately an average of about 40-50 % increase in yield of Pods (fruit).

Experiment No. 3. Another legume crop selected for the present study was cowpear (*Vigna sinensis*). *Rhizobium* sp. which was isolated from cowpea root nodules, already growing in the farm, was used in the preparation of biofertilizer. About 200 seeds were dressed with biofertilizer and were allowed to dry. These were sown in an experimental plots of 20' x 20'. One more bed of the same dimensions was used for control experiment. Random samples of ten plants each from control as well as treated one were taken out between 30-45 days for necessory observations. The data thus obtained is incorporated in Table 3.

The control plants of cowpea were showing less bacterial nodules as compared to the treated. On the average about 40-50 percent more nodulation was obtained in the treated plants (Fig. 5). In addition, treated plants were found comparatively greener, healthier and taller. In the control yellow colouration was more prevalent.

Experiments on cowpea have also been conducted at P.C.S.I.R. Model Farm, Malir on an area of about 5 acres.

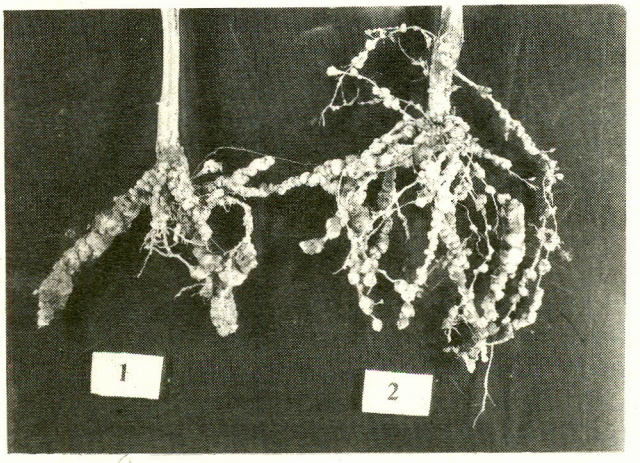

Fig. 5. Cowpea: Control roots showing smaller number of nodules (1) as compared to the treated (2).

DISCUSSION

Present studies on gram and cowpea provided encouraging results, the details of which are presented in respective Tables, and suggest that by dressing the seeds with biofertilizers, healthy plants with increased yield could be obtained. Experiments on gram plant as represented by Table 1 include observations made on the number of bacterial nodules after 45 days in the control as well as those treated with different concentrations of the bacterial preparations. It can be seen that in the control, none of the plants had any bacterial nodulation, whereas, in the treated ones nodulation occured and was related to the bacterial concentration in the biofertilizer. Ater 60 days (Table 2) the number of nodules on gram plants ranged from 14 to 36 in the control, whereas, the range in the treated was 32 to 72. This shows the efficacy of biofertilizers in promoting nodulation, and consequently, effective nitrogen fixation.

Similarly, nodulation in cowpea plants (Table 3) was found comparatively less in the control as compared to the treated. In the control the nodulation range was 20-61 whereas in the treated 56-104.

In addition to 10-15 days early flowering in both the crops, seeds treated with biofertilizers produced healthier plants with greener leaves and increased yield.

Table 1.

S.No. of plants	Name of Cros crop	Date of sowing	Date of observation & total duration	Source of bacteria	Type of soil pH	No. of nodules/plant			
						Control	1:1	1:2	1:3
1.	Gram (Sindhi white) *(Cicer arietinum)*	2.11.1981	17.12.1982	Gram nodules	Sweet earth	0	4	2	4
2.						0	3	4	3
3.			45 days		7.0	0	3	3	5
4.						0	2	4	4
5.						0	1	2	6

Table 2.

S.No.	Crop	Date of sowing	Date of observation and total duration	Source of bacteria	Type of soil pH	No. of nodules and pods/plants			
						Control		Treated	
						Nodules	Pods	Nodules	Pods
1.	Gram (Sindhi white) *(Cicer arietinum)*	9.12.1981	8.2.1982	Gram nodules	Sweet earth	14	37	32	84
2.						18	63	72	86
3.			60 days		7.0	20	58	40	172
4.						15	70	35	94
5.						21	60	42	105
6.						31	101	45	150
7.						19	90	38	148
8.						32	170	36	162
9.						36	111	40	170
10.						34	103	41	142

Table 3.

S.No.	Crop	Source of bacteria	Total duration	No. of nodules/plant	
				Control	Treated
1.	Cowpea (Lobia) *(Vigna sinenis)*	Cowpea nodules	Between 30 and 45 days	61	104
2.				41	95
3.				20	97
4.				34	101
5.				42	85
6.				45	87
7.				54	74
8.				31	69
9.				20	71
10.				41	56

The production of maximum number of nitrogen fixing nodules and the yield increase in gram (40-50 %) is considered a direct consequence of biofertilizer. To sum up the discussion, it would be relevant to stress the importance and future scope of biofertilizers for Pakistan in view of the renewable resources, high costs of chemical fertilizers, salinity and pollution problems.

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