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Effect of Some Rhizosphere Fungal Flora on the Productivity of Some Crop Plants

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Abstract: The experiments were performed with a motive of having maximum crop in minimum time and cost. The interactions of mycorrhizal forms and root of crop plants are many-a-times beneficial. Plants like *Cajanus cajan* L., *Glycine max* L., *Cymopsis tetragonoloba* L. and *Abelmoschus esculentus* L. are the crops with importance to small farmers of India. The cultures of rhizosphere fungi, in questions, were obtained by routine culture methods on PDA. With the help of soxhlet, Extract of the fungi selected, were prepared in water. The studies promise that the role played by mycorrhizal organism is helping to increase the yield, in minimum period. In all the experiment's yield increased significantly with virtually no hazardous effects and with alarmingly low cost. Such experiments will surely help hawkers-cum-farmers make both ends meet. Different concentrations aim at the significant results in vegetative and reproductive growth as Compared to control.

Key words: Rhizosphere fungi, crop plants, mycorrhizal forms

INTRODUCTION

Soil is a panorama of interactions between microbes and higher plants. Many early workers have significantly pointed out the importance of relations of rhizosphere and host plants. Generally host responses are the net results of activity of mycorrhizal associates.

Use of chemical fertilizers- virtually blindly has made it difficult to make both ends meet for bulk of Indian farmers. Not only the prices of chemical fertilizers are skyrocketing but also constant use of the same is instrumental in disturbing the fertility of soil sometimes beyond repairs. Ubiquitous nature of microbes has lured to explore the possible role of these microbes as biofertilizers.

Roots are the sites to provide nutrient supply for wide range of micro-organisms. Earanna *et al.* (1999) observed increased growth in *Coleus barbatus* due to AM-fungal inoculations. Fraechia *et al.* (2000) has demonstrated mutualistically beneficial interactions of soil micro-organisms. In addition to fungi, *Azolla* is also reported to be an effective bio-fertilizer by Kannaiyan (2000). Kumar and Kannaiyan (2001) have also reported *Azolla* to be instrumental in maintenance of soil fertility. The increase in growth and yield of various crops in response to mycorrhizal inoculations have already been reported by some workers Sailo and Bagyaraj (2003), Sumana and Bagyaraj (2003).

The quality and quantity of nutrients-organic materials in to the rhizosphere as exudates (non metabolic leakages) and secretions (metabolic releases), plant mucilage's etc. can change significant in response to variety and combination of factors and conditions. So tailoring, the growth and reproduction of the host plants was the goal of the experiments carried out.

MATERIALS AND METHODS

A team of workers carried out experiments since the year two thousand on different crops with interesting findings. The crops included in experiments are as under: local varieties of *Cajanus cajan* L., *Glycine max* L., *Cymopsis tetragonoloba* L. and *Abelmoschus esculentus* L., The selected rhizosphere microbes were *Aspergillus* and *Mucor* because of their constant association with host roots.

A general practice in Vidarbha (Maharashtra State, India) is to have mixed crop. Soil samples were collected from where the crop, in question, was harvested in just the previous season and were having the same crop in the present year also. The plants were carefully dug out. The roots with adhered soil were kept in bottles with proper indexing. The bottles, in questions, were shaken vigorously in sterile distilled water to prepare a suspension. This suspension was, then, used to have serial dilutions (Hildebrand, 1950). The last dilution was

utilized to obtain fungal colonies only after identification; auxenic cultures were obtained from such colonies by serial dilution technique on PDA to have sufficient crop. The cultures used in all these experiments were *A. niger*, *A. flavus* and *Mucor mucido*. The culture flasks were shaken, as per requirement, by using electric shaker for half an hour every alternate day.

The cultures were allowed to grow for 8 to 10 days, in liquid medium. The mat was filtered through Whatmann filter paper No. 42. The fungal mat on the filter paper was dried in oven at about 80-90°C. The filtrate along with unused medium was boiled for above one hour and stored at room temperature. Extract of the dried mat was prepared in sterile distilled water with the help of Soxhlet. (Subheder, 1993)

Before the treatment, the extract and the filtrate were added with sterile distilled water to make the concentrations up to 20, 30, 40, 60 and 80 ppm. The soil use for the requisite treatment was collected from different agricultural field where the requisite crop is cultivated regularly. Such soil was sterilized neatly in Autoclave at 15 pounds pressure. About 100 polythene bags were filled with sterilized soil and in each bags 3 to 5 seeds were sown. Thinning of plants was done as per the requirement of the crop under treatment. The control was watered only. The plants under treatment were supplied with solution of the extract and filtrate having different concentrations i.e. 20, 30, 40, 60 and 80 ppm. as the case may be. To acclimatize with natural conditions, the same experiment was repeated on agricultural land, at Amravati University Campus, Amravati i.e., *in vivo* in case of *Cajanus cajan* L., only.

RESULTS AND DISCUSSION

Recently Metwally (1988) has indicated effects of enhanced microbial populations of selected microbes in soil. Subhedar and Hande experimented with *Cajanus cajan* L., which is a common mixed crop in this area (i.e., Vidarbha, Maharashtra, India). Seeds treated with extract of *A. niger* culture germinated within eight days while the seeds treated with water only (i.e., control), consumed sixteen days for germination. The concentrations of the said extract were 20, 40, 60 and 80 ppm and were equally effective. (Fig. 1 and 2)

It is evident from Table 1, that the treated plants required about seventy two days to achieve height of more than forty cm. However control attained the height of 30 cm. in the same period (Fig. 3). In 136 days, treated plants exhibited buds or flowers (Table 1) while the control, vegetative growth only (Fig. 4). The treated plants developed pods in 144 days while control required 160 days (Table 1). In treated plants, yield was more than control (Table 1). It seems that the initial impetus received by the treated plants continued till the end. Hence, the treatment is instrumental in achieving maximum crop in minimum period.

Kundu and Gaur (1980a, b) have reported increase in yield of cotton and of wheat owing to *Aspergillus* activities. Nagaraju and Nanjundappa (1996) have also reported increase in yield of Cow pea owing to *Aspergillus*. Geeta Singh and Tilak (2002) have recommended use of mycorrhizal microbes either alone or in combination to be beneficial. So rhizosphere fungi and roots of host plants having mutually beneficial relationship. The present study is to

Table 1: Effect of extract of *A. niger* on development *Cajanus cajan* L. plant

No. of days	20 ppm conc.	40 ppm conc.	60 ppm conc.,	80 ppm conc.	Control
1	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown
8	Germinated	Germinated	Germinated	Germinated	Germinated
16	2.5 cm	7.5 cm	2.5 cm	2.5 cm	--
24	5 cm	12.5 cm	5 cm	5 cm	2.5 cm
32	10 cm	17.5 cm	7.5 cm	12.5 cm	7.5 cm
40	30 cm	35 cm	17.5 cm	15.0 cm	10 cm
48	37.5 cm	42.5 cm	22.5 cm	20.0 cm	15.0 cm
56	42.5 cm	50.0 cm	32.5 cm	30.0 cm	20.0 cm
64	52.5 cm	65.0 cm	37.5 cm	35 cm	22.5 cm
72	60 cm	70.0 cm	47.5 cm	42.5 cm	30.0 cm
80	65 cm	77.5 cm	60 cm	57.5 cm	37.5 cm
88	72.5 cm	100 cm	65 cm	67.5 cm	45.0 cm
96	82.5 cm	120 cm	82.5 cm	77.5 cm	52.5 cm
104	97.5 cm	127.5 cm	92.5 cm	87.5 cm	65 cm
112	107.5 cm	147.5 cm	110 cm	112.5 cm	75.0 cm
120	117.5 cm	165 cm	120 cm	120 cm	90 cm
128	135 cm	Buds	Buds	Buds	95 cm
136	Buds	Flowers	Flowers	Flowers	95 cm
144	Flowers	320-330 Pods*	290-300 Pods*	285-290 Pods*	Buds
152	280-290 pods*	--	--	--	Flowers
160	--	--	--	--	260-280 Pods*

* Total number of pods per plant



Fig. 1: Treated plants and control (*in vitro*) (after 20 days)



Fig. 2: Treated plants and control after 90 days (*in vitro*)



Fig. 3: Treated plants (*in vivo*) after 100 days



Fig. 4: Treated plants *in vivo* showing flowers not the control (marked)

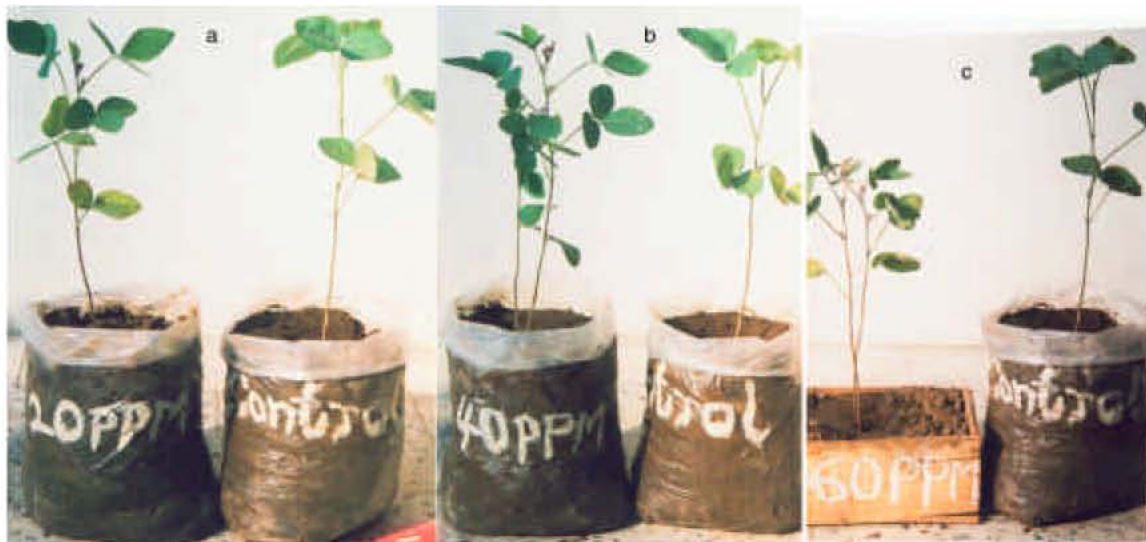


Fig. 5a-c: Treated plants with flowers and control without flowers



Fig. 6: Seeds of treated plants and control



Fig. 7: *Cymopsis* treated plants and control showing vegetative growth.



Fig. 8: Treated plants with pod and control without

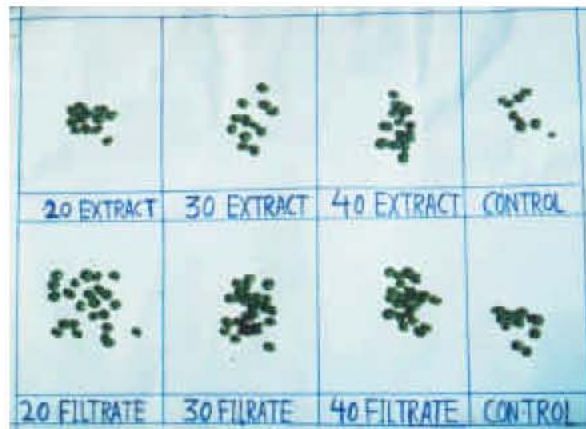


Fig. 9: Seeds of treated plants and control



Fig. 10a-c: Flowering and formation of pods in treated plants and control

Table 2: Growth Characteristics of soybean plant under various treatments

No. of days	20 ppm conc.	40 ppm conc.	60 ppm conc.	Control
1	Seed sown	Seed sown	Seed sown	Seed sown
3	Germinated	Germinated	Germinated	--
7	7 cm	8 cm	6.9 cm	Germinated
14	12.6 cm	12.9 cm	9.7 cm	8 cm
21	19 cm	20.2 cm	18 cm	17 cm
28	22 cm	25 cm	20 cm	18.2 cm
34	29.1 cm	38 cm	22 cm	23.5 cm
40	35 cm (Bud)	40 cm (Bud)	26 cm (Bud)	30 cm
48	37.6 cm Flowers	43 cm (Flower)	27.1 cm (Flowers)	31.2 cm
55	Flower 40 cm	Flower 47 cm	Flower 43cm	Bud (35 cm)
62	14 Pods	28 Pods	10 Pods	Flowers

Table 3: Effect of *A. flavus* on development of *Cymopsis* plant

No. of days	Extract			Filtrate			
	20 ppm	20 ppm	20 ppm	20 ppm	30 ppm	40 ppm	Filtrate
1.	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown
3.	G	G	G	G	G	G	-
5.	-	-	-	-	-	-	G
11.	5.5 cm	5 cm	4.5 cm	6 cm	4 cm	5 cm	3 cm
18.	7 cm	7 cm	5.5 cm	6.5 cm	6 cm	5.5 cm	4 cm
23.	8 cm	8 cm	8 cm	9 cm	7 cm	8 cm	4 cm
25.	Buds	Buds	Buds	Buds	Buds	Buds	-
29.	-	-	-	-	-	-	Buds
31.	Flower	Flower	Flower	Flower	Flower	Flower	-
36.	-	-	-	-	-	-	Flower

G - Germination

Table 4: Effect of treatment on flower, fruit and seed

No. of days	Extract			Filtrate			Control
	20 ppm	30 ppm	40 ppm	20 ppm	30 ppm	40 ppm	
36th	15 flowers	15 flowers	19 flowers	22 flowers	15 flowers	11 flowers	7 flower
40th	8 fruits	9 fruits	11 fruits	13 fruits	11 fruits	8 fruits	3 fruits
65th	9 seeds	6 seeds	8 seeds	7 seeds	6 seeds	7 seeds	3 seeds
	Total seeds	Total seeds	Total seeds	Total seeds	Total seeds	Total seeds	Total seeds
	72 (8 x 9)	54 (9 x 6)	88 (11 x 8)	91 (13 x 7)	66 (11 x 6)	56 (8 x 7)	9 (3 x 3)

explore the effect of metabolites of *A. niger* on *Cajanus cajan*. The qualitative and quantitative changes in the rhizosphere micro flora may be attributed to the alternation in the pattern of root exudates of *Cajanus cajan*. Naturally this act is having vice-versa implications too. The results are very promising (Table 1). Subhedar (1993) has also obtained virtually the same results while studying the same plant with the same fungal association. The local variety used by Subhedar was Gajra while Subhedar and Hande used the local variety named BDN-1.

Subhedar and Padwad (2003) studied effects of *A. niger* on the variety of soybean which was used by majority of farmers in this area i.e. Maha bij No. 335. The concentrations of extract used were 20, 40 and 60 ppm. As depicted in Table 2, germination in the treated seeds was observed in three days, while control required seven days, along with other characters when extract was given at fixed intervals. Within 40 days, flower buds appeared on all treated plants, however, control required 55 days for budding (Fig. 5a-c). The treated plants exhibited reproductive growth about 15 days early to control

(Table 2). The overall growth in 20 and 40 ppm on the 14th days was promising as compared to the growth observed in 60 ppm and control. The total number of pods present per plant in 20 ppm were 14, in 40 ppm were 28 and in 60 ppm were 10 in the stipulated period (i.e., 62 days) while control was in flowering stage only (Table 2)

In 2004, Subhedar, Bhoge and Bawaskar have worked on the same lines on local variety of *Cymopsis* (variety-Gomati). Constant association of *A. flavus* and *Cymopsis tetragonoloba* L., plants was observed. The investigators have aimed at finding out effect of the activities of the associating partners, hence, effect of metabolites of the said fungus on the growth and productivity of host plants are studied. The concentrations under study were prepared as described under materials and methods. The plants under treatments were watered with 20, 30 and 40 ppm extract and filtrate for stipulated period (Fig. 6). Table 3 shows that the treated plants germinated on 3rd day while the control was on the 5th day. The Table 3 indicates that on the 18th day, plants treated with extract exhibited average

Table 5: Effects of extract and filtrate of *A. niger* and *M. mucido*

No. of days	20 ppm		30 ppm		40 ppm		Control
	Extract	Filtrate	Extract	Filtrate	Extract	Filtrate	
1	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown	Seed sown
3	Germination	Germination	Germination	Germination	Germination	Germination	Germination
4							Germination
8	4.7 cm	4.7 cm	4.5 cm	4.3 cm	4.5 cm	4.5 cm	3 cm
9	3rd leaf	3rd leaf	3rd leaf	3rd leaf	3rd leaf	3rd leaf	
10							3rd leaf
15	6.4 cm	4.8 cm	4.6 cm	4.4 cm	5.3 cm	4.6 cm	3 cm
27	8.3 cm	6.5 cm	7.5 cm	7.5 cm	7 cm	6.5 cm	5.5 cm
31	Flower	Flower	Flower	Flower	Flower	Flower	5.5 cm
36							Flower
39	3rd bud	3rd bud	3rd bud	3rd bud	3rd bud	3rd bud	2nd bud
40	Buds	Buds	Buds	Buds	Buds	Buds	Buds
42	Buds	Buds	Buds	Buds	Buds	Buds	Buds
46	Buds	Buds	Buds	First fruit	First fruit	Flower	Buds
48	First fruit	Buds	First fruit	Fruit	Fruit	First fruit	Buds
54	Fruit	First Fruit	Fruit	Fruit	Fruit	Fruit	Buds
65							First Fruit

Table 6: Number of seeds and fruits per plant

Total No. of fruits	20 ppm		30 ppm		40 ppm		Control
	Extract	Filtrate	Extract	Filtrate	Extract	Filtrate	
Fruits	10	10	9	10	10	9	8
Total No. of seeds	140	140	54	90	90	72	48
No. of seeds per fruit	14	14	06	09	09	08	06

height between 5.5 to 7 cm. while plants treated with filtrate were 5.5 to 6.5 cm and control growth up to 4 cm (Fig. 7). The Table 4 indicates that filtrate with 20 ppm is Producing highest number of flowers while extract with 40 ppm shows development of 19 flowers per plant on 36th day. As compared, control shows development of only seven flowers per plant. A general tendency observed in this plant: development of fruits is significantly less than the number of flowers produced per plant (Table 3), size and number of seeds per pod is also more than the control (Fig. 8 and 9). By this treatment, the marginal farmers cum hawkers will be benefited though on the fringe.

Subhedar and Bissa in 2005 alongwith Dharkar experimented on the local variety of lady's finger with combination of two rhizosphere fungi. The extract and filtrates of combination of *A. niger* and *M. mucido* exhibited promising results (vide Table 5) The dilutions of extract and filtrate used were 20, 30 and 40 ppm concentration by adding requisite amount of distilled water. Healthy seeds of a local variety of lady's finger-Ankur-40 were sown in polythene bags along with control.

The experimental set included hundred plants along with control. In this experiment the treated seeds germinated on the 3rd morning and the control consumed 4 mornings. The plants under treatment required 31 days for flowering while control required 36 days

(Table 5; Fig. 10a). Normally the local varieties of lady's finger required 65 days to fruit while plants under treatment bore fruits within 46 days (Table 5). The number of seeds is significantly different in treated plants (Table 6).

CONCLUSIONS

“Mother nature is very generous”. Many microbes inhabit soil and are helpful in the growth and development of plants. The investigators feel that chemical fertilizers are not only costly but hazardous also. India being a country of farmers, with small farms, majority of farmers finds it very difficult to make both ends meet. It is therefore, an attempt to search a method which will suffice the requirement of hawker cum-farmers and will not pollute or disturb the “Mother nature”. One can observe that the method discussed involves low costs, easy handling and maximum crop in minimum period along with virtually no hazardous degradation of soil.

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