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Study of the progression of bacteria in rhizosphere of *Coriandrum sativum* grown in the arid region like Solapur

Bapat Tripti[1], Mane Vishwadeep[2], Rakshe Virbhadhra[3], Nakka Pooja[4], Koli Kartiki[5], Kallurkar Sakshi[6], Mule Rohini[7] and Madgundi Linal[8]

[1] *Associate professor, Department of Microbiology, D.B.F. Dayanand College of Arts and Science, Solapur Maharashtra,*

[2,3,6] *Department of Microbiology, D.B.F. Dayanand College of Arts and Science,*

[4,5,7,8] *Department of Microbiology, D.B.F. Dayanand College of Arts and Science, Solapur, Maharashtra*

Abstract

The Rhizosphere of any plant is a dynamic environment. An enormous number of microbial activities are continuously going on in this particular region as a result of diversity of microorganisms. It is a micro-biome. It is an active region of soil, often rich in organic matter and other nutrients. The microorganisms play an important role in the improvement of soil fertility and recycling of nutrients. The microbial diversity depends upon the type of plant, soil environment and other physical factors. However, the type and the number of microorganisms change with the time and also it is dependent upon the developmental stage of the plant. Each plant shows a peculiar rhizosphere microflora. Each and every microorganism of this region is carrying out some specific activity. The present study is taken to determine the variation and progression of heterotrophic bacteria in the rhizosphere of Coriandrum sativum. It is a preliminary study done by undergraduate students with the limited resources and hence is restricted to the rhizosphere bacterial count which can grow on nutrient agar. The study not only reflected bacterial diversity but clearly indicated the progression in bacterial count throughout the developmental stages of plant. Thus, this clearly indicates microbial ecological succession.

Keywords- Coriandrum sativum, rhizosphere, progression, micro-biome, diversity

I. INTRODUCTION

Microorganisms play an important role in the soil environment. They make the soil fertile due to their tremendous oxidation capacity. They are also involved in carrying out a variety of biochemical reactions and in regulation of various biogeochemical cycles. The soil is very rich environment and thus favors existence of diverse populations. The microbial activities are controlled by various physical, chemical and biological factors. Amongst all, the most important are the ecological interactions in the microbial population. These interactions have direct effect on the growth of specific type of organism.

The bacteria that are reported to be always there in rhizosphere of *Coriandrum* include the genera like *Bacillus, Arthrobacter, Hafnia, Planococcus, Micrococcus, Pseudomonas and Serratia*.

The study of microbial diversity can be done by culturing techniques and some advanced molecular biology techniques. The limitation to the cultural techniques is that they don't cover non-culturable organisms. The present study is taken to count the bacteria from rhizosphere of *Coriandrum* which are present from the time of seed germination and up to the harvest of plant. Considering the great degree of diversity the study is restricted to aerobic bacteria. The study included growing of *Coriandrum* in different soils of arid region and counting the colony number on Nutrient agar. The colony count obtained during the period of seed germination to harvest showed remarkable progression. In the early period some variation in type was observed. However, with the development of the plant the diversity in bacterial population became insignificant and the soil

environment seemed to be dominated by almost a single genus.

Coriandrum is purposely chosen for the experiment as it grows fast in any type of soil. It is a delicious herb that adds flavor to the Indian cuisine.

II. MATERIALS AND METHODS

A. Testing some basic parameters of soil:

1) For particle size -soil, sieves (2.0 mm, 0.5mm)

A fixed quantity of all 7 soil samples (50 gm) is crushed to break the clods. It is then sieved through a 2.00 mm sieve. This removes coarse sand and fine sand is sieved out. Fine sand is further sieved through 0.5 mm sieve. The fraction thus obtained is clay and silt mixture. All the contents separated are weighed separately for each sample.

2) For water holding capacity -distilled water, funnel, beaker, filter paper

It is measured by preparing a mixture of 20 gm of soil in 20 ml of distilled water and filtering it through a filter paper and the quantity of the filtrate obtained is measured. The water holding capacity is calculated by the following formula:

WHC (%) = [20 ml (D/W) - filtrate (ml)] /wt of soil (20 gm)

3) For pH – soil dilutions, pH paper

Soil suspension is prepared and pH is checked by using pH paper.

4) Ca and Mg content (EDTA titration method) -Ammonium acetate solution, 1 N NaOH, Murexide indicator, 0.01M EDTA solution, Ammonium buffer, Erichrome black indicator, Conical flasks, beakers, burette, pipette

For Ca and Mg content estimation, 1 gm of soil is weighed and mixed with 1 ml ammonium acetate solution. The mixture was kept overnight. On the next day it was decanted and filtrate was diluted to 200 ml. Of which 20 ml filtrate was mixed with 0.4 ml NaOH .To the mixture 20 mg of murexide indicator was added and the solution was titrated against 0.01 M EDTA solution. Endpoint is pink to purple.

For only Ca estimation, the above mentioned ammonium acetate dilution is used .To the 2 ml of ammonium acetate solution,1 ml distilled water , ammonium buffer and 3 -4 drops of Erichrome black T were added and titrated against 0.01 M EDTA solution. Endpoint is wine red color to blue.

As Mg alone can't be estimated, Both Ca and Mg are together estimated first and then the value of Ca estimation is subtracted from the Ca + Mg estimation to estimate Mg.

Following formula is used to calculate Ca content:

$$\text{Ca (meq/100g)} = \frac{A \times 400.8 \times 200 \text{ ml(total volume of soil extract prepared)}}{10 \text{ ml (volume of soil extract titrated)} \times 20.04 \times 10 \times \text{wt. of soil (1 gm)}}$$

Following formula is used to calculate Mg content:

$$\text{Mg (meq/100g)} = \frac{B\text{-}A \times 400.8 \times 200 \text{ ml (total volume of soil extract prepared)}}{2 \text{ ml (volume of soil extract titrated)} \times 1.645 \times 12.16 \times 10 \times \text{wt. of soil (1 gm)}}$$

(A=volume of EDTA for Ca determination in ml,
B= volume of EDTA for Ca+Mg determination in ml, meq=equivalent weight)

5) Carbon content (Walkley and Black method) -1N $K_2Cr_2O_7$ solution, conc.H_3 PO_4,, Diphenylamine indicator, 0.5N ferrous Ammonium sulfate(FAS) solution.

0.5 gm of sieved soil was added to 10 ml $K_2Cr_2O_7$ and 20 ml H_2SO_4 .It was kept for 30 min. The mixture was filtered and diluted to 200 ml. To the mixture 1 ml H_3PO_4 and1 ml diphenylamine indicator was added. It was then titrated against 0.5 N FAS solution. Endpoint was black blue to

brilliant green color. Organic Carbon content was estimated by following formula:

$$\% \text{ organic C} = (B-T) \times 0.5 \text{ N FAS} \times 0.003 \times \frac{100}{0.5 \text{ gm (Wt. of the soil)}}$$

(B= volume of 0.5 N FAS required for Blank titration, T=volume of 0.5 N FAS required for sample)

B. For bacterial count-Soil sample, sterile Nutrient agar plates, sterile dilution blanks of distilled water, sterile pipettes, spreader, alcohol

The rhizosphere and non rhizosphere bacterial count was done by spread plate method. For this soil from rhizosphere and non rhizosphere zone was collected. Then the dilutions were prepared by using sterile distilled water.0.1 ml of 10^{-3} dilution was spread on three sterile Nutrient agar plates. The same procedure was repeated for non rhizosphere soil. However, the procedure for rhizosphere bacterial count was repeated daily in triplicates. The time period selected for the count was from the seed germination up to the harvest. For the results an average colony count was taken into consideration.

C. Biochemical tests

1) Starch hydrolysis test- Sterile Starch agar plates, Iodine solution

Isolated colonies were spot inoculated on sterile starch agar plates and incubated. The amylase production activity/starch hydrolysis was determined by flooding the plate with Iodine solution and the plate was observed for a clear zone around the colonies.

2) Gelatin hydrolysis test-Sterile Gelatin agar plates, 1% Tannic acid

Isolated colonies were spot inoculated on sterile Gelatin agar plates and incubated. The gelatinase production activity/Gelatin hydrolysis was determined by flooding the plate with 1% Tannic acid solution and the plate was observed for a clear zone around the colonies.

3) IAA production test -Salkowasky's reagent (0.5 N $FeCl_3$ and 35 % perchloric acid), bacterial cultures in nutrient broth tubes (4 tubes with Tryptophan and 4 tubes without tryptophan), centrifuge, D/W, pipettes, test tubes

Suspensions of isolated colonies were inoculated in sterile Nutrient broth tubes sets. One set contained simple Nutrient broth and other set contained nutrient broth along with Tryptophan amino acid as a precursor. The tubes were incubated and on the next day they were centrifuged at 3,000 RPM for 15 min. In a test tube, 1ml supernatant, 1ml distilled water and 1 ml Salkowasky's reagent were added and observed for pink color development. Above procedure was followed for all the colonies inoculated in their respective nutrient broth tubes sets (both).

4) Phosphate solubilization test-Sterile Pikovasky's agar plates, bacterial culture suspensions

Isolated colonies were spot inoculated on sterile Pikovasky's agar plates and incubated. The plates were observed for clear zone of phosphate solubilization around the colonies.

5) Oxidase test- Oxidase reagent (NNN'N'-Tetramethyl-p-phenyldiamine dihydrochloride), bacterial colonial growth, filter paper.

For this test the Oxidase reagent is taken on a filter paper and the colonies are rubbed on the paper. If the organism is Oxidase positive then the color changes to purple on the filter paper.

6) Catalase test- Hydrogen peroxide, bacterial colonial growth

For this test a drop of hydrogen peroxide is flooded on the bacterial growth. If effervescence develops then the test is positive.

III. RESULTS AND DISCUSSION

Table 1. Physico-chemical parameters of soil

Sr.no.	Sample code	pH	Water holding capacity	Particle size[gm] (In 50gm Soil)			Ca (meq/100g)	Mg (meq/100g)	C (%)
				CS	FS	S+C			
1.	VM	6	0.625	30.54	19.46	5.96	640	125.2	0.50
2.	VR	6	0.70	29.5	20.5	9.42	560	100.8	0.19
3.	PN	7	0.50	23.02	17.44	10.54	479	70.12	0.58
4.	KK	6	0.625	28.07	22.93	8.75	800	125.2	0.50
5.	SK	6	0.625	28.53	21.47	14.13	719	120.2	0.40
6.	RM	2	0.76	26.87	23.13	8.87	400	120.2	0.39
7.	LM	6	0.77	30.56	19.44	9.54	640	135.2	0.39

(CS-Coarse sand, FS-Fine sand, S+C-Silt + Clay, meq-equivalent weight)

Table 2. Bacterial count

Sr.no.	Sample code	In unsowed soil[A] (Count/gm)	In Non-Rhizosphere Soil after sowing[B]	Rhizosphere effect(A/B)
1.	VM	$6.12x\ 10^6$	3.37×10^6	1.816
2.	VR	5.40×10^6	2.68×10^6	2.0149
3.	PN	5.02×10^6	3.36×10^6	1.494
4.	KK	2.79×10^6	1.55×10^6	1.8
5.	SK	3.16×10^6	1.33×10^6	2.375
6.	RM	2.34×10^6	1.71×10^6	1.368
7.	LM	5.21×10^6	2.88×10^6	1.8090

Table 3. Rhizosphere bacterial count on Nutrient Agar plates

Colony count/gm of soil sample=mean count (per 100 mg) X dilution factor (10^3) X 10(factor to convert into gm)

Sr. no	Sample code	Days---- (count X 10^6/gm of soil)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.	VM	6.80	4.28	2.50	2.89	7.08	4.46	6.12	6.18	6.26	6.45	6.80	7.16	7.48	7.60	8.09
2.	VR	8.50	7.70	9.35	4.76	6.76	5.43	5.40	5.20	5.69	5.92	6.12	7.41	8.14	8.60	9.26
3.	PN	1.81	1.77	1.80	3.72	5.55	3.63	5.02	4.95	5.53	6.25	6.43	7.13	8.05	7.98	8.85
4.	KK	1.42	1.28	1.33	1.42	2.17	2.90	2.79	2.77	3.73	4.00	4.41	4.63	4.95	5.20	5.80
5.	SK	0.93	0.98	1.20	1.40	2.06	2.36	3.16	3.30	4.02	4.24	5.66	7.43	7.63	7.83	8.16
6.	RM	1.29	3.90	2.07	2.97	3.17	4.01	2.34	5.16	5.10	4.34	4.95	5.34	6.88	7.00	9.12
7.	LM	2.10	2.56	2.06	3.16	4.10	3.15	5.21	3.74	3.26	3.54	5.51	6.31	7.17	8.09	8.80

Table 4. Diversity in bacterial population

Sr.no.	Colony code	DAYS TILL DIVERSITY WAS OBSERVED(count x 10^6/gm of soil)						
		1	2	3	4	5	6	7
1	LY	0.32	0.25	0.22	0.14	0.08	2	-
2	MY	0.12	0.09	0.05	0.02	0.01	-	-
3	GOLDEN	1.81	2.10	3.69	4.51	6.56	7.00	8.91
4	WHITE	0.85	0.92	1.63	2.00	1.51	2.56	3.25

Table 5. Colony characters of isolated colonies

Sr. no	Colony code	Size (mm)	Shape	Color	Margin	Opacity	Elevation	consistency	Gram nature	motility
1.	LY	3.5	circular	Lemon yellow	Regular	Opaque	Low convex	Smooth	**Gram positive (cocci)**	motile
2.	MY	3	circular	orange	Regular	Opaque	Flat	Smooth	**Gram Negative (short rods)**	motile
3.	GOLDEN	3	circular	Golden brown	Regular	Translucent	Flat	Smooth	**Gram Negative (rods)**	motile
4.	WHITE	4	circular	White	Irregular	Opaque	Convex	dry	**Gram positive (rods)**	motile

Table 6. Biochemical analysis of isolated colonies

Sr. no	Colony code	Gelatin hydrolysis	Starch hydrolysis	IAA production		Catalase	Oxidase	Phosphate solubilization
				Tryptophan added	Without Tryptophan			
1.	**LY**	-	+	-	-	+	-	-
2.	**MY**	+	-	+	+	+	-	-
3.	**GOLDEN**	-	-	+	-	+	+	-
4.	**WHITE**	-	+	-	-	+	+	-

('+' indicates positive result and '-'indicates negative result)

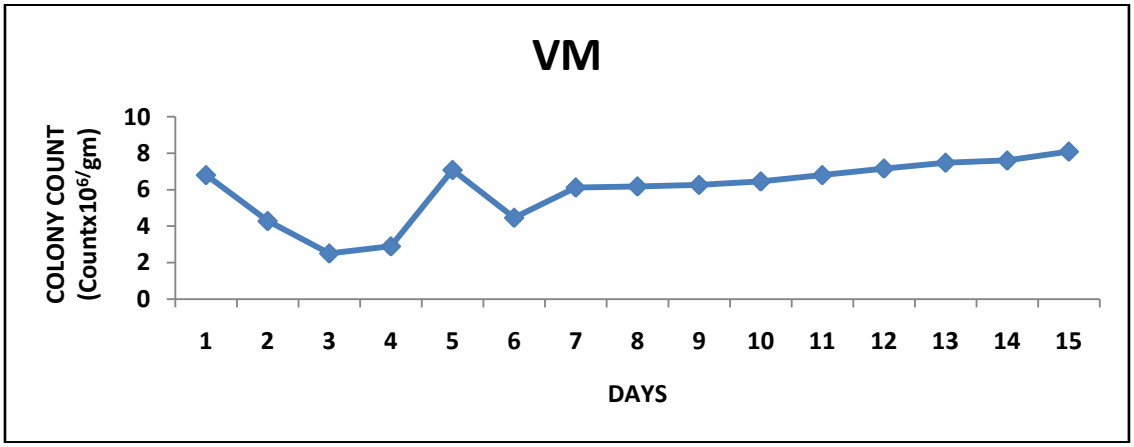

Figure 1. Colony count and progression trend in sample VM

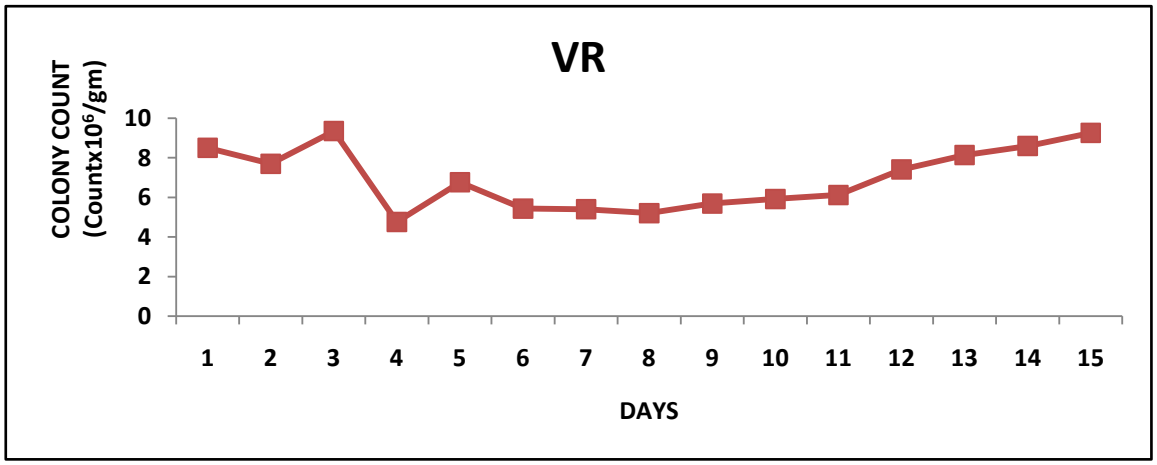

Figure 2. Colony count and progression trend in sample VR

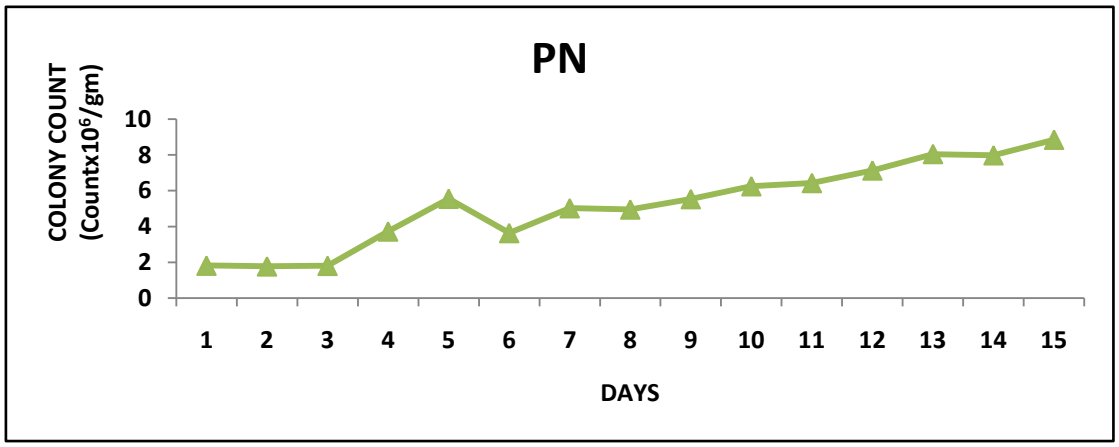

Figure 3. Colony count and progression trend in sample PN

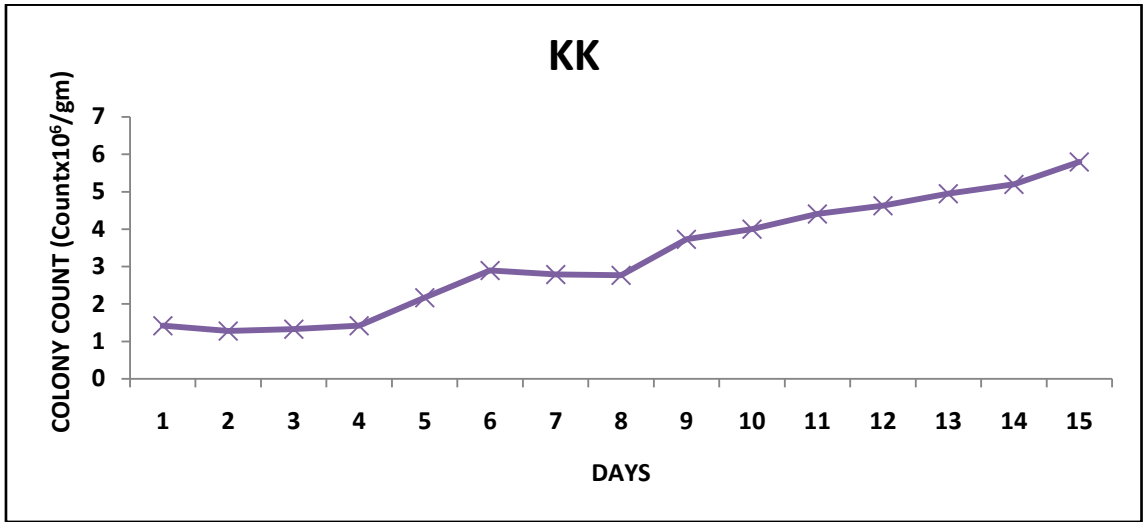

Figure 4. Colony count and progression trend in sample KK

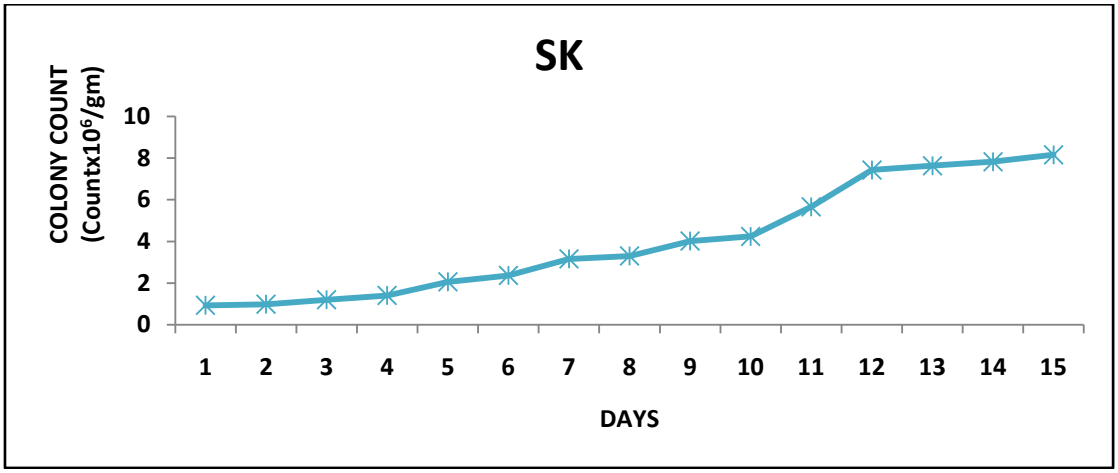

Figure 5. Colony count and progression trend in sample SK

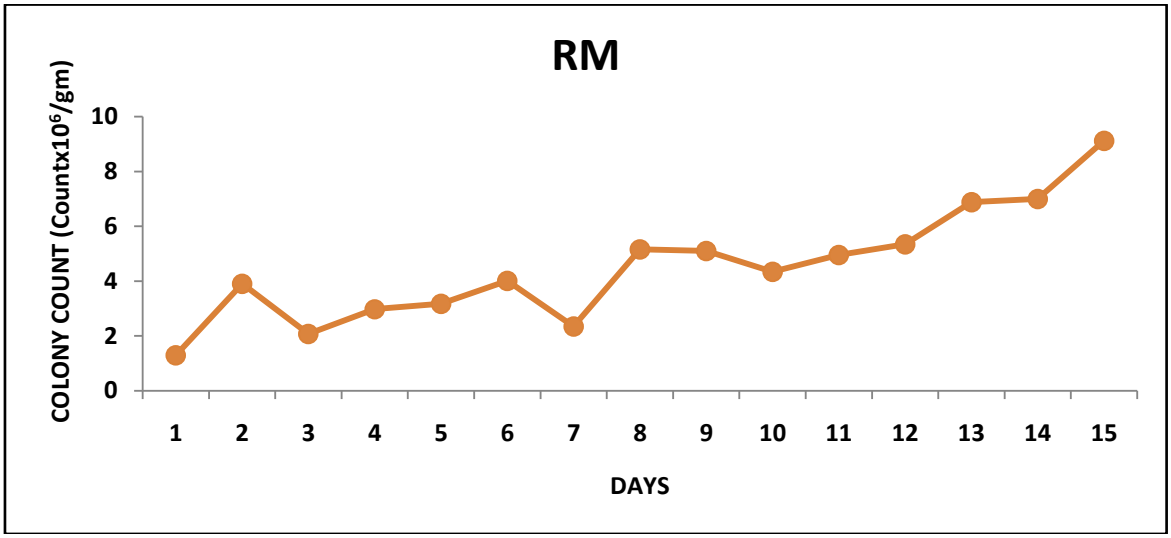

Figure 6. Colony count and progression trend in sample RM

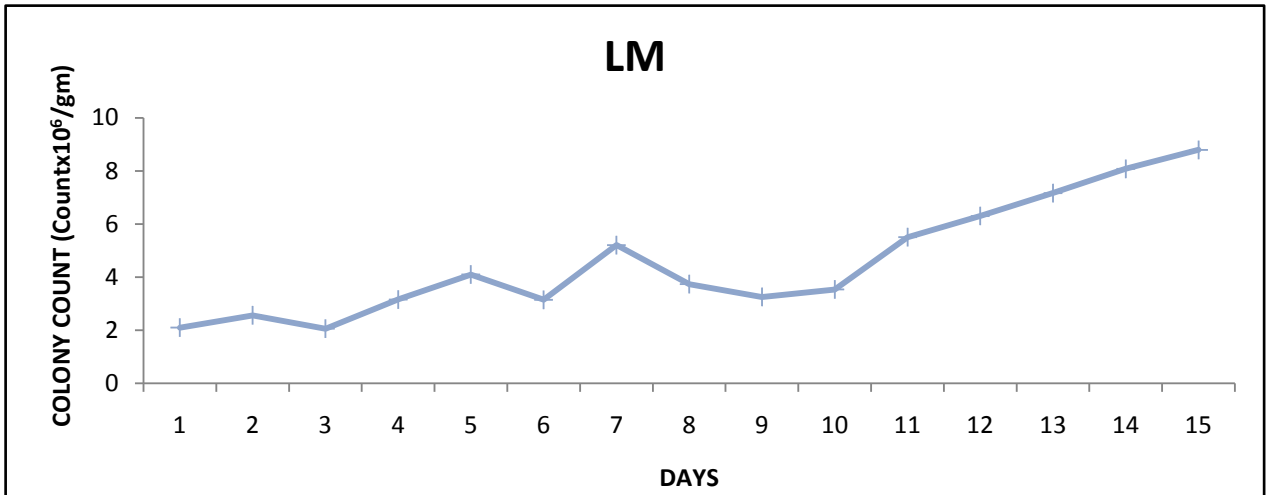

Figure 7. Colony count and progression trend in sample LM

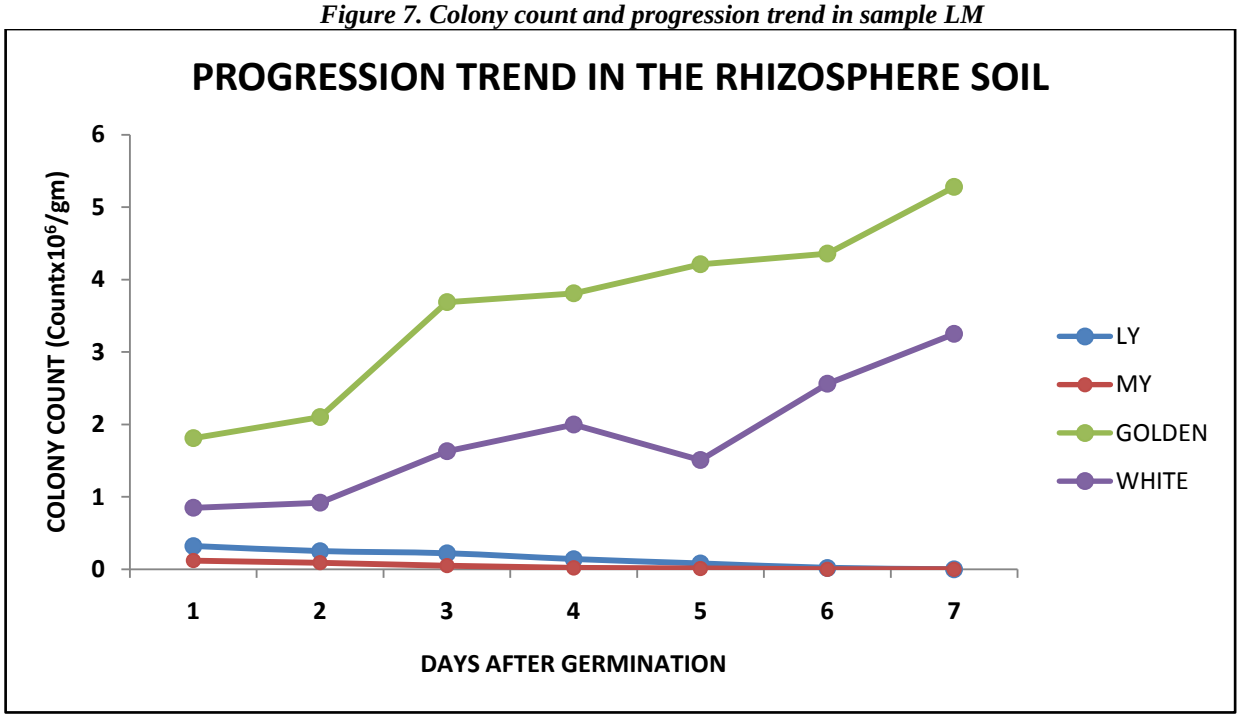

Figure 8. Progression trend in rhizosphere soil

The study on the progression of rhizospheric heterotrophic bacteria in Coriandrum sativum resulted in some interested findings. It clearly demonstrated presence of diverse bacterial population in the early stages of growth while only single dominance in the latter stages of growth i.e. at harvest.

Let us discuss more about the results.

The soil was analyzed before sowing for a few physical and chemical parameters (Table 1) .The experiment was carried out by a group of students residing in different parts of the city, hence a little variation was found in above said parameters.

But average findings are --pH was found out to be 6.0,water holding capacity was in the range of 0.5-0.77,the particle size for coarse sand 23.02-30.56,for fine sand 17.44-23.13 and for clay and silt mixture 5.96-14.13.The Ca content was between 400-800 meq /100 gm, Mg content between 70.12-135.2 meq /100 gm and Organic Carbon content between 0.19-0.50%.

The bacterial count taken before sowing i.e. for unsowed soil was in the range of 2.34-6.12 x 10^6 per gram of soil. The bacterial count for non-rhizosphere region after sowing was found in the range of 1.33-3.37 x 10^6 per gram of soil (Table 2).The average bacterial count taken for each sample code in the period from seed germination to harvest showed a clear-cut progression i.e. increase in

colony count with the developmental stages of the plant.(Table 3).The day 1 recorded in tables and graph is actually the day of seed germination .The results recorded in the tables and the corresponding graphs drawn for each soil sample can be interpreted as follows :

For sample code VM progression in colony count was observed from day 3 to day 15. (Figure 1)
For sample code VR progression was observed from day 6 to day 15. (Figure 2)
For sample code PN progression was observed from day 8 to day 15. (Figure 3)
For sample code KK a clear-cut progression was observed from day 2 to day15. (Figure 4)
For sample code SK again a clear-cut progression was observed from day 1 to day 8 and then from day 11 to day 15. (Figure 5)
For sample code RM a delayed progression was observed from day 10 to day 15. (Figure 6)
For sample code LM a progression was observed from day 9 to day 15. (Figure 7)

Almost each and every sample showed progression in bacterial count.

The interesting part of this experiment is that bacterial diversity was observed in early stages of growth which was not so conspicuous in the latter period of growth i.e. from day 7 of seed germination. Interestingly we could find the presence of two types of organisms, one of which was not so dominant but showed its existence (Table 4 and figure 8).

We have identified these cultures on the basis of morphology, Cultural properties and few differential and promising biochemical properties. (Table 5, 6)

IV. CONCLUSION

Rhizosphere of any plant is dynamic and diverse environment. The diversity involves limitless interactions. Our results reflected not only the diversity in bacterial population but also an increase in the bacterial count i.e. Progression with the progress of development of plant growth. The limitations for performance restricted us to count heterotrophic bacteria. But surely we have succeeded in finding the progression in the rhizosphere bacterial count of Coriander.

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