THE ASIAN JOURNAL OF HORTICULTURE
Volume **9** | Issue 1 | June, 2014 | 143-146
e ISSN- 0976-724X | Open Access-*www.researchjournal.co.in* |

Research Paper

Article history **:**
Received : 14.12.2013
Revised : 30.04.2014
Accepted : 09.05.2014

Effect of bio-fertilizers on general and beneficial microbial population in the rhizosphere of garden rue (*Ruta graveolence* Linn.)

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ABSTRACT : A pot experiment was carried to study effect of bio-fertilizers on general and beneficial microbes in the rhizoshere of garden rue plants. Three bio-fertilizers such as *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescence* were in liquid formulation as single, dual and triple inoculation by dipping roots of seedling for up to 20 minute before transplanting. Results revealed that, inoculation of bio fertilizers significantly increased both beneficial and general microbial population in rhizosphere of garden rue. Among all the inoculation dual and triple inoculation of bio-fertilizers were recorded maximum CFU g^{-1} soil with respect beneficial and general micro-flora except fungi and actinomycetes.

KEY WORDS : Biofertilizers, Microbial population, Rhizosphere

HOW TO CITE THIS ARTICLE : Vijendrakumar, R.C. and Shankarappa, T.H. (2014). Effect of bio-fertilizers on general and beneficial microbial population in the rhizosphere of garden rue (*Ruta graveolence* Linn.). *Asian J. Hort.*, **9**(1) : 143-146.

Garden rue (*Ruta graveolens* Linn.), a member of the family Rutaceae, have commercial and medicinal importance. Garden rue is locally known as *Nagadalisoppu* or *Sannisoppu* in Kannada (Kapoor,1996) and garden rue, is a native of Balkan Peninsula and South Eastern Europe. *Ruta* is a genus of strong scented, erect, glabrous herbs or under-shrubs of 30-90 cm height distributed throughout the Mediterranean region and Temperate Asia.

Bio-fertilizers like *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescence* is known to improve the soil fertility, crop productivity as well as establishment of seedling in several crops through atmospheric nitrogen fixation, solubilizing inorganic and organic phosphorus and other nutrients, by increasing in multiplication rate under the rhizosphere of the plants (Verma, 1993; Subba Rao, 1995). Hence, in the present investigation, an attempt has been made to the study the influence of bi-fertilizers in single, dual and triple inoculation to the roots of the plants by dipping in liquid formulation on increasing in the general and beneficial population of microbes in the rhizosphere of the garden rue plants.

RESEARCH METHODS

The general microbial population *viz*., bacteria, fungi and actinomycetes and beneficial microflora *viz*., phosphate solubilizing bacteria (PSB), *Pseudomonas fluorescens* and *Azospirillum* sp. of the rhizosphere of garden rue plants was estimated at 60 days after planting and at harvest (120 DAP) of garden rue in the Department of Microbiology, University of Agricultural Sciences, Bengaluru, during the year 2011-12.

Garden rue plants were inoculated with different liquid bio-fertilizers in single, dual and triple. The liquid bio-fertilizers like *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens* (20× 108 cfu ml^{-1}) were obtained from the Caryogen Biotech. Pvt. Ltd. University of Agriculture Sciences, Dharwad. 45 days old seedlings selected from nursery of the Medicinal crops section, GKVK, Bangalore and inoculated the seedlings by dipping root system in liquid formulation for 20 minute after that seedlings were planted in poly bags contain a media comprised of sand, soil, FYM in ratio of 2:1:1 and kept in the field with 60 × 60 cm spacing. Irrigation and intercultural operations were taken up and harvest was done after 120 days of planting. The experiment was laid out in a Completely Randomized Design with three replications.

Experiment had eight treatments comprised of control, single, dual and triple inoculation of liquid bio-fertilizers such as T_1-*Azospirillum lipoferum*, T_2-*Pseudomonas striata*, T_3-*Pseudomonas fluorescens*, T_4–*Azospirillum lipoferum* + *Pseudomonas striata*, T_5–*Azospirillum lipoferum* + *Pseudomonas fluorescens*, T_6-*Pseudomonas striata* + *Pseudomonas fluorescens* and T_7-*Azospirillum lipoferum* + *Pseudomonas striata* +*Pseudomonas fluorescens.*

The soil samples from each treatment were collected separately, at 60 days after planting and at harvest, from vicinity of roots or soil which was attached to the roots of garden rue plants and pooled treatment wise, homogenized and used for microbial population estimation. Serial dilution plate count technique was used for estimation microbial population by collection of ten grams of pooled soil (treatment wise) was mixed in 90 ml sterile water blank to give 10^{-1} dilution. Subsequent tenfold serial dilutions up to 10^{-6} were made by transferring serially 1 ml of the dilution to 9 ml of sterile water blanks. 1 ml dilution of 10^{-6} for bacteria, 10^{-4} for actinomycetes and 10^{-3} for fungi, phosphate solubilizing bacteria and *Pseudomonas fluorescens* dilutions were transferred to a sterile Petridish and approximately 15 ml of pre-sterilized, cooled molten media *viz*., Soil Extract Agar, Martins Rose Bengal Agar, Kurster's Agar, Pikovoskay's medium and king's 'B' medium were poured for estimating soil bacteria, fungi, actinomycetes, phosphate solubilizing bacteria and *Pseudomonas fluorescens*, respectively. The plates were rotated twice in clockwise and anticlockwise direction for proper mixing of the inoculums. After solidification of the media, plates were kept for incubation in an inverted position at 30 ± 1^0 C for a week time and emerged colonies were counted.

Serial dilution most probable number technique was used for estimation of *Azospirillum* sp. by collection of ten grams of pooled soil (treatment wise) was mixed in 90 ml sterile water blank to give 10^{-1} dilutions. Subsequent dilutions up to 10^{-6} were made by transferring serially 1 ml of the dilution to 9 ml of sterile water blanks. 1ml of 10^{-3}, 10^{-4} and 10^{-5} dilutions were transferred to 5 test tubes of three sets containing approximately 6 ml of pre-sterilized, cooled semisolid Dobereiner media for estimating *Azospirillum* sp. Then test tubes were closed with cotton plugs and kept for incubation at 30 ± 1^0 C for a week time. Number of positive tubes (growth occurred) and negative tubes (no growth occurred) were counted and values expressed according to Cochran (1950) standard table fixed for estimating the most probable number method for microbial population by the help of positive tubes.

The microbial population data collected from the each treatment were averaged and Completely Randomised Design (CRD) was employed to find out the significance among different treatments with the help of 'F' test (Sunderaraju *et al*., 1972).

RESEARCH FINDINGS AND DISCUSSION

The results obtained from the present investigation are summarized below :

General microbial population in the rhizosphere of garden rue:

The microbial population of bacteria were significantly recorded highest at 60 and 120 DAP due to inoculation of bio-fertilizers. The maximum population of bacteria was recorded in the triple inoculation of *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* (T_7-51.67× 10^{-6} and 83.67 × 10^{-6} cfu g^{-1} soil) which was at par with T_6 and T_4 and the minimum population was recorded in the control (Table 1). Increased bacterial population in the rhizosphere of plants inoculated with bio-fertilizers is because of the triple and dual inoculation bacterial cultures that would have proliferated in rhizosphere of garden rue. Hence, in all the stages of plant growth, the microbial population with respect to bacteria has given significant values. In case of fungi and actinomycetes population, there was no effect of bio-fertilizers at both the stages of plant growth. However, with respect to fungi, highest population was recorded in triple inoculation T_7 (8.67× 10^{-4} cfu g^{-1} soil) which was at par with control at 60 DAP. At 120 DAP, highest population of fungi was also recorded in triple inoculation treatment T_7 (12.33× 10^{-4} cfu g^{-1} soil) and minimum in control, with respect to actinomycetes in T_5 (*Azospirillum* + *Pseudomonas fluorescens*) and T_7 was recorded highest at 60 and 120 DAP, respectively. Similar findings with respect to total bacteria were found in jasmine (Manonamani, 1992) and Paramaguru and Gopi (2006), who reported the synergestic interactions between introduced microbial inoculants and the native microorganisms in the rhizosphere of plants.

Beneficial microbial population in the rhizosphere of garden rue:

There was a significant increase in the population of *Azospirillum* sp., PSB and *Pseudomonas fluorescens* in all the bio-fertilizers inoculated plants than the control (Table 2). The maximum population of *Azospirillum* sp was recorded in dual and triple inoculation (11.33× 10^{-3} cfu g^{-1} soil and 29.00 × 10^{-3} cfu g^{-1} soil at 60 and 120 DAP, respectively). *Azospirillum* population was increased at harvest (120 DAP) than at 60 DAP, in all the treatments. However, minimum population was recorded in control at both the stages of plant growth. Inoculation of *Azospirillum lipoferum* resulted in the better colonization of *Azospirillum* population. Increase in rhizosphere microflora may be attributed to the multiplication of the strains in the rhizosphere, utilizing root exudates produced by the plants (Shivakrishnaswamy, 2001) and synergestic interactions between introduced microbial inoculants in dual and triple and also the native

Table 1 : Effect of bio-fertilizers on general microbial population (cfug^{-1} soil) in rhizosphere soil of garden rue (*Ruta graveolens* Linn.) at 60 and at 120 days after planting (at harvest)

Treatments	Total bacteria (cfu x10^{7})		Total fungi (cfu x10^{4})		Total actinomycetes (cfux10^{3})	
	60 DAP	120 DAP	60 DAP	120 DAP	60 DAP	120 DAP
T_1-*Azospirillum lipoferum*	27.33	48.67	7.33	9.67	11.33	13.00
T_2-*Pseudomonas striata*	23.00	43.67	5.67	11.33	11.00	12.00
T_3-*Pseudomonas fluoresceas*	28.00	46.33	8.00	9.67	11.67	15.67
T_4-*Azospirillum lipoferum* –*Pseudomonas striata*	42.00	83.67	6.33	10.00	12.33	15.33
T_5-*Azospirillum lipoferum* –*Pseudomonas fluorescens*	49.33	74.00	7.33	11.00	12.67	15.33
T_6-*Pseudomonas striata* +*Pseudomonas fluorescens*	50.33	80.33	7.67	12.33	10.00	11.00
T_7-*Azospirillum lipoferum*+*Pseudomonas striata* +*Pseudomonasfluorescens*	51.67	83.00	8.67	10.67	11.33	16.33
T_8-control (uninoculated)	21.33	34.67	8.67	9.00	10.33	15.00
S.E. ±	2.26	2.87	0.74	0.92	0.84	1.21
C.D. (P=0.05)	9.33	11.89	NS	NS	NS	NS
C.V (%)	10.69	8.07	17.30	15.36	12.86	14.79

DAP-Days after planting NS-Non significant

Table 2 : Effect of bio-fertilizers on beneficial microbial population (cfug^{-1} soil) in rhizosphere soil of garden rue (*Ruta graveolens* Linn.) at 60 and 120 days after planting (at harvest)

Treatments	*Azospirillum* sp. (cfu x 10^{3})		Phosphate solubilizing bacteria (cfu x 10^{3})		*Pseudomonas fluorescens* (cfu x 10^{3})	
	60 DAP	120 DAP	60 DAP	120 DAP	60 DAP	120 DAP
T_1-*Azospirillum lipoferum*	8.73	24.67	13.33	16.67	10.00	13.33
T_2-*Pseudomonas striata*	4.83	13.67	20.00	36.67	13.33	16.67
T_3-*Pseudomonas fluoresceas*	5.10	13.33	13.33	23.33	13.33	26.67
T_4-*Azospirillum lipoferum*+*Pseudomonas striata*	11.33	25.00	16.67	25.00	10.00	16.67
T_5-*Azospirillum lipoferum* –*Pseudomonas fluorescens*	9.40	25.67	10.00	16.67	16.67	33.33
T_6-*Pseudomonas striata* +*Pseudomonas fluorescens*	7.33	19.33	23.33	36.67	23.33	26.67
T_7-*Azospirillum lipoferum* +*Pseudomonas striata*+*Pseudomonas fluorescens*	9.43	29.00	20.00	33.33	13.33	30.00
T_8-Control (uninoculated)	4.57	9.03	10.00	13.33	6.67	10.00
S.E. ±	1.479	1.783	1.178	2.12	1.76	1.44
C.D. (P=0.05)	6.11	7.36	4.86	8.77	5.27	5.96
C.V (%)	33.75	15.28	12.89	14.59	22.96	11.53

DAP-Days after planting

microorganisms in the root zone of crop. The present investigations were in conformity with the results obtained by Rajadurai *et al.* (2000) in african marigold. With respect to PSB (P-solubilizing bacteria), *Pseudomonas striata* had recorded maximum population in dual inoculation of *Pseudomonas striata* + *Pseudomonas fluorescens* (23.33 × 10^{-3} cfu g^{-1} soil and 36.67 × 10^{-3} cfu g^{-1} soil at 60 and 120 DAP, respectively) and minimum was in control. Similarly, for *Pseudomonas fluorescens*, maximum population was recorded with dual inoculation of *Pseudomonas striata* + *Pseudomonas fluorescens* (23.33 ×10^{-3} cfu g1soil at 60 DAP) and dual application of *Azospirillum lipoferum* + *Pseudomonas fluorescens* (33.33 × 10^{-3} cfu g^{-1} soil at harvest). Increased microbial population in dual application may be due to intraction effect of two microbes to each other that is earlier what we say it will be synergetic effect of micro organism and also the same findings accordance with Savitha (1996) in chick pea and Yadav and Singh (1990) in sugarcane.

Conclusion:

It was concluded that general and beneficial microbial population was more in dual and triple inoculated plants of garden rue than single and uninoculated plants. So dual and triple inoculation of bio-fertilizers were more effective in increasing the population of general and beneficial microbes in the rhizosphere of plants ultimately leads to better growth also occur in plants.

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