Plant Archives Vol. 14 No. 1, 2014 pp. 171-175 *ISSN 0972-5210*

EFFECT OF LIQUID BIO FERTILIZERS ON GROWTH, YIELD AND SURVIVAL OF SEEDLINGS IN GARDEN RUE (*RUTA GRAVEOLENS* LINN.)

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Abstract

A pot experiment was conducted to study the effect of different liquid bio fertilizers on growth, yield and survival of seedlings in garden rue (*Ruta graveolens* Linn.). Liquid bio fertilizers *viz.*; *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens* were used to treat root system of seedlings in single and combination before transplanting. The results revealed that, the treatments differed significantly with respect to growth, yield and survival of seedlings. Among the various treatments tried seedlings treated with the combinations of *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens* recorded highest values for plant height (51.40cm), number of branches (13.27), number of compound leaves (44.50), stem girth (8.27mm) and fresh bio-mass yield (73.73g) compare to single inoculation and control.

Key words : *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens*.

Introduction

Garden rue or common rue or Herb-of-Grace, botanically called as *Ruta graveolens* Linn. (family Rutaceae) is one of the important medicinal plant attaining high medicinal importance in recent times. The entire plant is of medicinal importance and has used to cure various ailments like kapha and vata, strangury, fever, flatulence and epilepsy, fresh plant act as scorpion and insects repellent, leaves and seeds boiled in olive oil and the mixture is rubbed for rheumatism pains and swellings (Vaidyaratnam, 1996) in the tradition system of medicines since ancient times.

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. Garden rue, is a native of Balkan Peninsula and South Eastern Europe, which is sturdy perennial plant with much branched rounded stem. Leaves alternate, shortly petioled, ultimate segments oblong to spathulate, leaflet with numerous translucent small short stalked oil glands containing a very irritating volatile oil, have a strong, disagreeable odour and bitter, acrid, pungent taste; flowers small, 12 mm in diameter, yellowish in corymbs, strongly aromatic; petals 4-5, spoon shaped with dentate or wavy margin; capsules small with lobes somewhat rounded (Prajapathi *et al*., 2003).

The plant contains pilocarpine, which is used to induce abortion in horses. The fresh herb on steam distillation yields a pale or greenish volatile essential oil (0.06%) called rue oil, the oil is rich in methyl ketone (80-90%) and is used for the preparation of methyl-n-nonyl acetaldehyde, widely used as a synthetic perfume and also nowadays from rue plants, many number of products like Rue gel, Rue tea, Human growth complex, and *Ruta graveolens* capsules have been produced by the drug industries.

It is a seed propagated crop and hence, there is great demand for seed materials in order to optimize production of garden rue for the supply of raw materials to the drug industries. But, poor germination, slow growth and survival of seedlings have rendered the rapid multiplication and production of sufficient herbage for the drug industries. The modern and intensive agriculture methods are not

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only costly, but also cause soil and water pollution. Thus, by considering the recent concept of eco friendly technology, application bio fertilizers in single and combination substitute the above need in many crops.

The bio fertilizers are primary active strains of microorganisms, they are used either to fix atmospheric nitrogen or to solubilize plant nutrients, like phosphates, or to other wise stimulate plant growth through synthesis of growth promoting substances. Among biofertilizers *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens* are commonly used. *Azospirillum lipoferum* is a free living nitrogen fixing bacterium, *Pseudomonas striata* is a phosphate solubilizing bacteria help to solubilize insoluble inorganic phosphate such as rock phosphate, tri calcium phosphate, iron and allumonium phosphate and *Pseudomonas fluorescens* a plant growth promoting rhizobacteria cum bio-control agent, they were tested in single and in combinations on garden rue.

Thus, keeping the above points in view, the present study was undertaken to study the effect of liquid bio fertilizers as supplement of nutrient sources on growth, yield and survival of seedlings in garden rue.

Materials and Methods

The present investigation was carried out in Sanjivini Vatika, Medicinal crop section, respectively at Division of Horticulture, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bengaluru, during 2011-12. The Division of Horticulture is located at an altitude of 930 meters from mean sea level, at a latitude of 12^0 58 North and a longitude of $77^0$35 East. Mean maximum temperature during the period of experiment was ranged from 28.0^{0}C to 34.6^{0}C, while the mean minimum temperature ranged between 14.3^{0}C to 21.2^{0}C. Similarly, the maximum relative humidity of 73 per cent was recorded during July and lowest of 57 per cent was recorded in March. So also, the rainfall varied from 8.6mm (April) to 95.8 mm (July) 2012.

The experiment was laid out in a Completely Randomized Design with three replications. Experiment had eight treatments comprised of control, single and combination of liquid bio fertilizers such as $\mathbf{T_1}$- *Azospirillum lipoferum*, $\mathbf{T_2}$- *Pseudomonas striata*, $\mathbf{T_3}$- *Pseudomonas fluorescens*, $\mathbf{T_4}$–*Azospirillum lipoferum* + *Pseudomonas striata*, $\mathbf{T_5}$– *Azospirillum lipoferum* + *Pseudomonas fluorescens*, $\mathbf{T_6}$ - *Pseudomonas striata* + *Pseudomonas fluorescens* and $\mathbf{T_7}$- *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens*. The liquid bio fertilizers like *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens* (20 × 10^8 cfu ml^{-1}) are obtained from the Caryogen Biotech. Pvt. Ltd. University of Agriculture Sciences, Dharwad (Karnataka), India. 45 days old seedlings selected from nursery of the Medicinal crops section and treated the seedlings by dipping root system in liquid bio-fertilizers 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 timely harvest was done after 120 days of planting. Observations were recorded monthly for vegetative parameters like plant height, number of leaves, stem girth, fresh weight, dry weight by keep the seedlings in hot air oven at the temperature of 60^{0}C till constant weight was attained for up to 120 days after planting. The survival percentage of seedlings was also studied by counted the established seedlings from of the seedlings transplanted to the polybag in each of the treatment and their survival percentage was worked out. The data collected from the five labelled seedlings in 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).

Results and Discussion

In general various growth parameters including plant height, number of branches, number of leaves and girth of the seedling and yield parameters like fresh and dry weight of herbage and roots per plant and total biomass herb yield had increased due to the application of bio-fertilizers both in single and in combination compared to untreated plants.

Influence of various bio-fertilizers on growth parameters

Inoculation of bio-fertilizers influenced morphological characters of plants, resulting in their improved growth and development (table 1).

The combined application of bio-fertilizers resulted in increased plant height over the control and single inoculums at all the four stage of plant growth. The $\mathbf{T_6}$ with combination of *Pseudomonas striata* + *Pseudomonas fluorescens* had recorded highest plant height (25.23 cm and 32.47 cm at 30 and 60 DAP, respectively) and $\mathbf{T_7}$- combination with *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* had resulted in enhanced plant height (41.87 cm and 51.40 cm at 90 and 120 DAP, respectively). This might be due to increased cell elongation and cell

Table 1 : Influence of bio-fertilizers on vegetative parameters at 30, 60, 90 and 120 days after planting (at harvest) in garden rue (*Ruta graveolens* Linn.).

Treatments	Plant height(cm)				Number of branches				Number of compound leaves				Stem girth of plant (mm)			
	30DAP	60DAP	90DAP	120DAP	30DAP	60DAP	90DAP	120DAP	30 DAP	60DAP	90DAP	120DAP	30DAP	60DAP	90DAP	120DAP
T_1	21.50	28.97	39.37	47.80	4.27	5.20	7.73	12.07	14.47	25.60	33.33	41.73	3.33	4.40	6.07	7.47
T_2	24.17	27.63	36.20	41.47	3.67	5.13	7.33	11.27	13.53	23.67	29.73	36.07	3.40	4.53	5.83	7.20
T_3	21.73	26.67	33.83	38.83	3.33	4.47	6.87	10.27	13.67	21.87	29.40	34.67	3.33	4.40	5.20	6.93
T_4	21.40	27.60	41.60	48.97	3.73	5.27	7.60	11.20	13.33	25.73	34.73	40.73	3.07	4.20	5.47	7.07
T_5	22.63	28.87	40.60	49.27	3.80	5.47	7.87	11.60	13.33	24.13	34.13	40.30	3.20	4.27	5.47	7.40
T_6	25.23	32.47	41.67	48.93	4.07	5.53	7.60	12.60	14.40	27.80	35.33	43.67	3.47	4.67	5.80	7.60
T_7	24.43	32.30	41.87	51.40	4.07	5.80	8.00	13.27	14.00	29.13	35.80	44.50	3.47	4.53	6.27	8.27
T_8	17.90	23.90	30.23	34.37	3.13	4.13	4.67	7.27	10.67	16.67	23.67	31.13	2.83	3.47	4.40	5.80
S.Em±	1.33	1.68	2.61	3.33	0.22	0.31	0.63	0.92	1.75	2.33	2.35	2.87	0.22	0.24	0.35	0.41
C. D. at 5%	4.02	5.06	7.85	9.99	0.66	0.93	1.89	2.77	NS	6.99	7.05	8.60	NS	0.72	1.059	1.25
C V (%)	10.37	10.25	11.88	12.81	10.18	10.507	15.15	14.33	22.66	16.61	12.73	12.71	12.01	9.66	11.02	10.008

Treatments- 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*, **T_7**- *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* and **T_8**-control (uninoculated); **DAP-** Days after planting, **NS-** Non significant.

multiplication coupled with enhanced nutrient uptake by plants due to bio-fertilizer inoculation. The inoculation of *Azospirillum lipoferum* and P-solubillizing bacteria (PSB) probably caused the increased plant height in jasmine (Preethi *et al.*, 1999), this can also attributed to the production of plant growth promoting substances like IAA and GA in the vicinity of roots by inoculated microorganisms (Amitha and Savalagi, 2004). Application of *Azospirillum lipoferum* (**T_1**) had resulted in highest number of branches (4.27 at 30 DAP). At 60, 90 and 120 DAP the application of bio-fertilizers like *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* (**T_7**) in combination was recorded highest number of branches (5.80, 8.00 and 13.27, respectively) and minimum was recorded in the control. Increased number of branches were noticed in bio-fertilizers treatments could be attributed to activation of bio-active substances in plants due to the application of bio-fertilizers, which in the turn would have increased the availability of nutrients. Similar observations were recorded by Ajimuddin *et al.* (2005) in sweet basil.

There was a marked increase in the number of compound leaves per plant due to the bio-fertilizers treatment. Maximum number (14.47) of compound leaves per plant was recorded in the **T_1** (*Azospirillum lipoferum*) at 30 DAP. With respect to 60, 90 and 120 DAP number of compound leaves was recorded maximum in treatment combination of *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* (29.13, 35.80 and 43.567, respectively) and minimum in control. Generally, the plants which received combined application of bio-fertilizers produced the maximum number of compound leaves which could be mainly attributed to better growing conditions that prevailed in the vicinity of root zone and availability of more nutrients produced by the bio-fertilizers through fixation of nitrogen and solubility of phosphorous in the soil. These results are in accordance with that of Rajamanickam (2011) in *Mentha arvensis*.

The maximum stem girth was recorded in combined application of *Pseudomonas striata* + *Pseudomonas fluorescens* (4.67 mm) at 60 DAP and the application of *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* had recorded highest stem girth (3.47 mm, 6.27 mm and 8.27 mm at 30, 90 and 120 DAP, respectively) and minimum was in control. Similarly, present results were associated with Abd-El-Hadi Nadia *et al.* (2009) in spear mint (*Mentha viridis* L.).

The highest survival (100%) of seedling was recorded in the treatment combination of *Azospirillum lipoferum* + *Pseudomonas striata* (**T_5**) and *Azospirillum*

Table 2 : Influence of bio-fertilizers on survival of seedlings, fresh and dry weight of herb and roots and total biomass per plant at 120 days after planting (at harvest) in garden rue (*Ruta graveolens* Linn.).

Treatments	Survival (%)	Fresh weight of herb (g)	Dry weight of herb (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Total biomass weight (g)
T_1	97.78	61.33	11.87	7.73	1.93	69.07
T_2	95.55	60.53	12.53	7.87	2.23	68.40
T_3	97.78	56.73	10.27	6.60	1.73	63.30
T_4	93.33	62.80	11.73	9.07	2.03	71.87
T_5	100.00	61.47	12.27	8.80	2.63	70.27
T_6	95.55	63.87	12.67	9.67	2.97	73.53
T_7	100.00	65.07	12.80	8.33	2.73	73.73
T_8	86.66	41.80	9.40	5.53	1.60	47.20
S.Em±	2.07	3.49	0.67	0.72	0.25	3.76
C D at 5 %	6.23	10.45	2.02	2.16	0.77	11.28
C V (%)	3.75	10.202	10.02	15.76	20.04	9.702

Treatments-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*, **T_7**- *Azospirillum lipoferum* + *Pseudomonas striata* + *Pseudomonas fluorescens* and **T_8**- control (uninoculated).

lipoferum + *Pseudomonas striata* + *Pseudomonas fluorescens* (**T_7**) which was *on par* with **T_1**, **T_3** and **T_6** and minimum was in control (86.66%). Highest survival of the seedlings may be due to the application of bio-fertilizers that helps in the well establishment of root system in soil which leads to better establishment of seedling than the plants which have not received any bio-fertilizers (control). The present findings are in accordance with results of Rajamanickam *et al.* (2011) in *Mentha arvensis.*

Influence of various bio-fertilizers on yield parameters

The data (table 2) showed effect of bio-fertilizers in combination was found to increases fresh and dry herb weight significantly. The maximum fresh and dry weights were recorded with the application of *Azospirillum lipoferum* + *Pseudomonas striata* and *Pseudomonas fluorescens* (**T_7**-65.07 g and 12.80 g, respectively). Whereas, in control minimum fresh and dry weight of herb (41.0 g and 9.40 g, respectively) was recorded. Plant growth promoting factors like IAA, GA and Cytokinin like substances are known to be produced by PSB and *Pseudomonas fluorescens* (Gowda, 2004 and Jagadish, 2006). IAA is known to be involved in root initiation, cell division and cell enlargement (Salisbury, 1994), which may cause enhanced root growth and uptake of nutrient, this would lead to increase in the growth of herb and its weight. Similar results were reported by Kourosh *et al*. (2011) in *Ocimum basillicum*.

The highest fresh and dry weight of roots was recorded in the combination of *Pseudomonas striata* + *Pseudomonas fluorescens* (9.67 g and 2.97 g, respectively) and minimum in control. This increment may be due to the application of P-solubilizers in combination that enhances the P-solubilization in soil, which leads to more availability of phosphorous to the plant and ultimately increased root growth by increasing with phosphorous content in soil. Similar findings were recorded by Gajbhiye and Deshmuk (2010) in ashwagandha.

The highest biomass yield was recorded in the treatment combination of *Azospirillum lipoferum* + *Pseudomonas fluorescens* + *Pseudomonas striata* (73.73 g) and minimum in the control. Application of bio-fertilizers might have helped to increase the nutrient uptake in the inoculated treatments. Similar observations were recorded by Charan Kumar *et al.* (2012) in stevia.

Conclusion

From the present investigation, it was concluded that application of bio-fertilizers, in the treatment combinations of *Azospirillum lipoferum*, *Pseudomonas striata* and *Pseudomonas fluorescens* recorded maximum plant growth, yield and survivability of garden rue seedlings than single application and untreated.

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