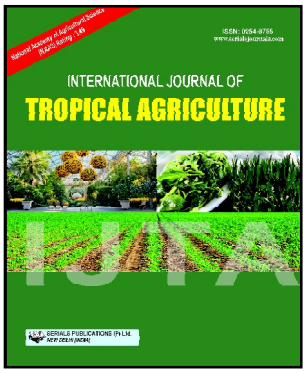

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"Studies on Methods of Application of Liquid Biofertilizers in Marigold (*Tagetes erecta* L.)"

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Abstract: The present investigation entitled "Studies on methods of application of liquid biofertilizers on Marigold (*Tagetes erecta* L.)" was carried out at the College of Agriculture, Pune-05. The objectives of the present research were to find out the beneficial and effective method of application of liquid biofertilizers and their effect on growth parameters and nitrogen uptake by marigold crop. In all there were sixteen treatments including *Azotobacter* cultures with recommended dose of fertilizers, 100% N, P and K, 75% N, 100% P and K, and absolute control replicated twice in Completely Randomized Design.

The application of these liquid biofertilizers (*Azotobacter*) showed significantly better soil microbial and chemical properties as well as improved crop growth than lignite based *Azotobacter* culture. Generally liquid biofertilizer (*Azotobacter*) proved to be effective over the control. The treatment T1 : Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A. with recommended dose of fertilizer recorded significant increase in plant height (50.50cm), number of branches (25.34), number of flowers per plant (40.60), yield of flowers per plant (122.13 g plant-1) and dry matter weight (44.67g plant-1) over all other treatments. It was followed by Liq. *Azo*.S.T. +100% R.D.N., Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A.+ 75% R.D.N., Liq. *Azo*.S.T.+ 75% R.D.N. The liquid biofertilizer (*Azotobacter*) also showed beneficial effects on soil chemical and biological properties. There was an increasing trend observed with respect to total *Azotobacter* population at flowering which was decreased at harvesting stage. The uptake of nitrogen was significantly highest under Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A. with recommended dose of fertilizer over rest of the treatments. Considering all these parameters, it is concluded that the application nitrogen fixing liquid biofertilizers (*Azotobacter*) as a S.T.+S.R.D.T.+S.A.+F.A. with 100% or 75% R.D.N. was found significantly superior than its carrier based counter parts and improved the soil biochemical properties as well as fulfilled the nutrient requirement of marigold crop to a considerable extent.

Key words: *Azotobacter,* liquid biofertilizers, Marigold

INTRODUCTION

Marigold (*Tagetes erecta* L.) occupies a prominent place in ornamental horticulture and is one of the commercially grown flower crops belonging to the family Compositae. Marigold is a heavy feeder of nutrients specially nitrogen and phosphorus (Nalwadi 1982). At present, these nutrients are supplied through chemical fertilizers. The continuous and discriminate use of chemical fertilizers leads to decrease in nutrient uptake and adversely affect the quality of produce (Agarwal, 2003). To overcome these problems biofertilizers can be good option in organic farming system to increase the crop yield and its quality without much investment of money and labour. Biofertilizer is commonly referred to a preparation that contains living microorganisms and it is expected that their activities will influence the soil ecosystem and produce supplementary substance for the plants (Parr *et al*., 2002).

Although lignite based biofertilizers are mostly being used for better crop production but its short shelf life of six months and poor quality and sensitivity to temperature has contributed for its failure in field. Lignite based biofertilizers having lack of identifiable character, lack of instant visual effects on application, unavailability of good carrier in local area, poor cell protection, poor moisture retention capacity, problem of proper packing, high transport cost, labour intensive are the reasons behind the failure of carrier based biofertilizers. Liquid biofertilizer technology is an alternative solution to lignite based biofertilizers.

Liquid biofertilizers comprises aids to preserving organisms, to delivering them to their targets and once there to improving their activities. By applying an appropriate liquid biofertilizers, the overall cost of production will be much lower as compare to traditional chemical fertilizers. The liquid biofertilizers also improve the soil quality and therefore the farmers can cut down the cost of soil maintenance, tremendously (Chin, 2010). Unlike the lignite based biofertilizers these liquid biofertilizers have a longer shelf life (Rao *et al*., 2004-07) and have lesser chances of contamination. Liquid biofertilizers have better survival on seeds and soil, no effect of high temperature, no loss of efficiency due to sub-culturing, cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport, easy to quality control, dosages are ten times less than lignite based biofertilizers and greater potentials to fight with native microbial population.

Owing to its ability to fix molecular nitrogen and therefore increase the soil fertility and stimulate plant growth, *Azotobacter* is widely used in agriculture (Narula *et al*., 2000). The cultures of *Azotobacter* synthesize considerable quantities of biologically active substances. Foremost among these are vitamins, nicotinic acid, indol acetic acid, gibberellins and biotin etc. *Azotobacter* has an ability to produce antifungal antibiotics and fungistatic compounds against pathogens *viz*. *Fusarium*, *Alternaria* and *Candida* (Mishustin and Shilnikova, 1972). Seed germination and vigour of the young plants was also observed to be improved due to *Azotobacter* inoculation (Mishustin and Naumova, 1962 and Shende *et al*. 1986). Particularly in nitrogen fixing liquid biofertilizers, liquid *Azotobacter* can save 10-15 kg of nitrogen per hectare. While the cost may seem to be higher, the shelf life is much higher than the lignite based fertilizers, thus making them economically viable. Generally liquid *Azotobacter* and other liquid biofertilizers are priced at Rs. 200 per litre which has a shelf life of one year. The lignite based ones cost Rs.30-40 per kg and last for about 5-6 months.

Various techniques have been introduced to produce biofertilizers, the concept of liquid biofertilizer originate from effective microorganisms (EM), which is available in liquid form (Higa and Parr, 1994). For this process bacteria are added to the routine culture medium and preservatives are

added to it. Thus the bacteria remain in a dormant state and remain viable for longer period and when added to the soil they become active. The density of free living nitrogen fixers such as *Azospirillium spp.*, *Azotobacter spp.* is increased in the rhizosphere than in the bulk soil. They contribute to nitrogen uptake by non-legumes in nitrogen deficient soils but due to intense competition for root exudates in the rhizosphere, their contribution to nitrogen uptake is likely to be small, however NPK-liquid contains cell protectant which does not allow root exudates to effect the bacteria. It is difficult to achieve the desired count in lignite based biofertilizers. The population density of these microbes is only 10^8 (10 crores) c.f.u./ ml. at the time of production and reduces day by day. While liquid *Azotobacter* count is as high as 10^9 c.f.u./ ml. which need to be maintained upto utility.Considering the diversified uses of liquid biofertilizers (*Azotobacter*) need was felt to study the use of liquid biofertilizers particularly for the benefit of the farmers. Therefore present investigation was carried out under the following.

MATERIAL AND METHODS

Material

Pots for glass house experiment

The earthen pots with 30 cm diameter and 30 cm height having capacity of 3 kg soil were used for conducting the pot culture experiment.

Disinfectant

Five per cent aqueous solution of copper sulphate ($CuSO_4$) was used for disinfecting the earthen pots.

Microbial inoculants

Liquid *Azotobacter* inoculants and lignite based *Azotobacter* inoculants were collected from BNF scheme, College of Agriculture, Pune 411005.

FYM

The well decomposed farm yard manure was obtained from Animal Husbandry and Dairy Science Department, College of Agriculture, Pune-411003.

Glassware's

The necessary branded glasswares *viz.* test tubes, petriplates, conical flasks, measuring cylinders, beakers, glass rods, pipettes, funnels, volumetric flasks, burettes etc. were used.

Equipments and other appliances

The laboratory equipment *viz.* autoclave, hot air oven, BOD incubator, laminar air flow cabinet, refrigerator, weighing balance, micro-kjeldhal's digestion unit etc. used whenever necessary.

Culture media

Jensen's medium was used for various purposes during investigation as specified in Appendix.

Miscellaneous material

Brown paper bags, test tube racks, micropipette, labels, meter scale, polythene bags, spirit lamp, plastic trays, inoculation needle, cotton, scalpel, sterilized water, distilled water etc. were used whenever necessary.

METHODS

Methods used for soil analysis

Table 1
Analysis of soil samples

	Biological properties		
1	Total *Azotobacter* count	Serial dilution and pour plate technique	Subba Rao (1999)

Methods used for plant analysis

Table 2
Analysis of plant samples

Sr. No.	*Parameter*	*Method*	*Reference*
1	Total nitrogen (%)	Kjeldhal's method	Parkinson and Allen,(1975)

Experimental details

Experimental site

Pot culture study was conducted in the glasshouse of All India Coordinated Cotton Improvement Project (AICCIP), Department of Plant Pathology and Agril. Microbiology, College of Agriculture, Pune-411005 and *in vitro* studies were carried out in laboratory of Biological Nitrogen Fixation Scheme, College of Agriculture, Pune - 411005.

Pot culture study

Treatment details

Design of experiment - Completely Randomized Design (CRD)

Test crop - Marigold (*Tagetes erecta* L.).

No. of treatments - 16

No. of replications - 2

Foliar application - 1.5 ml. for 100 ml. of water

Treatment details

GROUP A) With 100% R.D.N.

T_1 : Seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter*
(Liq. *Azo*.S.T.+S.R.D.T.+S.A.+F.A.)

T_2 : Seed treatment alone with liquid *Azotobacter*.
(Liq. *Azo*.S.T.)

T_3 : Seedlings root dipping treatment with liquid *Azotobacter*
(Liq. *Azo*. S.R.D.T.)

T_4 : Seed treatment + seedling root dipping treatment + soil Application with carrier based *Azotobacter*.
(Carr. *Azo*.S.T.+S.R.D.T.+S.A.)

T_5 : Seed treatment alone with carrier based *Azotobacter*.
(Carr. *Azo*.S.T.)

T_6 : Seedlings root dipping treatment with carrier based *Azotobacter* **(Carr. *Azo*. S.R.D.T.)**

T_7 : Foliar application **(Liq. *Azo*.F.A.)**

T_8 : Control. (No inoculation)

GROUP B) With 75% R.D.N.

T_9 : Seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter*
(Liq. *Azo*.S.T.+S.R.D.T.+S.A.+F.A.)

T_{10} : Seed treatment alone with liquid *Azotobacter*.
(Liq. *Azo*.S.T.)

T_{11} : Seedlings root dipping treatment with liquid *Azotobacter*
(Liq. *Azo*. S.R.D.T.)

T_{12} : Seed treatment + seedling root dipping treatment + soil Application with carrier based *Azotobacter*.
(Carr. *Azo*.S.T.+S.R.D.T.+S.A.)

T_{13} : Seed treatment alone with carrier based *Azotobacter*.
(Carr. *Azo*.S.T.)

T_{14} : Seedlings root dipping treatment with carrier based *Azotobacter* **(Carr. *Azo*. S.R.D.T.)**

T_{15} : Foliar application **(Liq. *Azo*.F.A.)**

T_{16} : Control. (No inoculation)

Seed inoculation

Commercial liquid *Azotobacter* inoculants of same growth phase were collected from BNF scheme, College of Agriculture, Pune-411005. Lignite based *Azotobacter* inoculant was also obtained from BNF scheme, Pune-411005.

For inoculation of liquid *Azotobacter*, the seeds of marigold were dipped in liquid *Azotobacter* suspension @3ml/kg of seeds. Seed dressing of *Azotobacter* culture was done @ 250 g/10 kg of seeds. Inoculated seeds were dried in shade before sowing.

Raising of seedlings

Seeds of marigold which were inoculated in liquid *Azotobacter* and lignite based *Azotobacter* inoculant as per treatment were sown in soil at a depth of 5 cm on raised seed bed. The seeds were covered with soil and soil was moistened with water.

Pot filling

The earthen pots used for pot culture experiment were surface sterilized with five per cent $CuSO_4$ and filled with soil and FYM in ratio of 2: 1 @ 3 kg/pot and same were marked according to treatments.

Fertilizers application

A fertilizer dose of 100:50:50 (N, P and K) was applied through straight fertilizer form i.e. Urea, Single Super Phosphate, Murate of Potash respectively. Half dose of nitrogen was applied at the time of transplanting and remaining half dose of N was given 30 days after transplanting.

Root dipping and transplanting seedlings

The healthy seedlings were uprooted from bed by giving light irrigation, washed with water and roots were dipped into liquid *Azotobacter* solution and *Azotobacter* inoculants and transplanted in earthen pots containing 3 kg soil. Normal cultural practices i.e. weeding, irrigation etc. were carried out. Two seedlings were transplanted per pot.

$$Germination\ percentage = \frac{Total\ number\ of\ seeds\ germinated\ per\ bed}{Total\ number\ of\ seeds\ sown}$$

Collection of soil samples for microbial analysis

The microbial count for total *Azotobacter* population in soil samples were recorded before sowing, at flowering, and at maturity stage of plant growth using serial dilution pour plate technique (Subba Rao, 1999). The soil was collected from rhizosphere of plant. The total *Azotobacter* population was enumerated on Jensen's medium (Appendix) at 10^4 dilution. The plates were labeled properly, incubated at 28 ± 2^0C temperature for 72 hours and colonies were counted.

The total *Azotobacter* population in 1 gram soil was calculated by following formula.

$$No\ of\ bacteria\ per\ gram\ of\ soil = \frac{Av.\ Plate\ colony\ count \times dilution\ factor}{Oven\ dry\ weight\ of\ 1g\ of\ sample}$$

Microbial count of different formulations used

The microbial count for total *Azotobacter* population of different *Azotobacter* formulations used were taken using serial dilution pour plate technique (Subba Rao 1999). The sample was collected from broth obtained from BNF scheme, Pune. The *Azotobacter* population was enumerated on Jensen's medium (Appendix). The plates were labeled properly, incubated at 28±2 °C temperature for 72 hours and colonies were counted. The count was taken at 10^9 dilutions for liquid culture and at 10^8 for lignite based culture.

Statistical analysis

The data obtained in different observations were computed statistically as per Completely Randomized Design (CRD) by using the standard statistical methods as described by Panse and Sukhatme (1967) for its statistical significance. The data were presented

in tabular form with suitable graphical illustrations and figures at appropriate places.

RESULT AND DISCUSSION

Total viable count of *Azotobacter* in different formulations under study

The data on *Azotobacter* population in different fresh formulations used for present study are presented in (Table 3). The *Azotobacter* count was recorded with liquid *Azotobacter* culture (22x 10^9 ml^{-1}) was higher than *Azotobacter* count (5x10^8 g^{-1}) in lignite based *Azotobacter* culture.

Tripathi and Ayyappan (2005) reported the higher population of *Azotobacter* as colony forming unit in water media in charcoal immobilized *Azotobacter* treatment than *Azospirillum* over alginate immobilization.

Table 3
Total viable count of *Azotobacter* in different formulations

Azotobacter culture	*Total Azotobacter count*
Liquid *Azotobacter* culture	22 (10^9 ml^{-1})
Lignite based *Azotobacter* culture	5 (10^8 g^{-1})

Effect of different *Azotobacter* formulations and their methods of application on germination and growth of marigold

Seed germination

The results in respect of germination of marigold seeds as influenced by different liquid *Azotobacter* formulations with their different methods of application are presented in (Table 4). Inoculation with liquid *Azotobacter* increased the marigold seed germination significantly over their respective lignite based *Azotobacter* culture and uninoculated control. It revealed that an application of liquid *Azotobacter* increased the seed germination from 74 to 96 percent.

The significantly higher germination percentage was recorded due to inoculation with liquid *Azotobacter* culture (96%) over all the treatments, followed by lignite based *Azotobacter* culture (88 %), and the least germination (74%) was recorded in uninoculated control.

Similar observations were noted by Dere (1986) in brinjal due to *Azospirillum* and *Azotobacter* inoculation and Sajindranath *et al.*(2002) reported in okra due to *Azotobacter* + PSB. Nagananda *et al.* (2010) reported that *Azotobacter* as a biofertilizer performed better than inorganic fertilizers in relation to seed germination of *Trigonella foenumgraecum* L.

Table 4
Effect of different *Azotobacter* formulations and their methods of application on seed germination of marigold

Treatments	*Germination %*
Liquid *Azotobacter* culture	96
Lignite based *Azotobacter* culture	88
Control	74

Plant height

The plant height of marigold recorded at 30, 60, 90 and 120 days after planting as influenced by application of different formulations of *Azotobacter* cultures with their different methods of application are presented in (Table 5).It revealed that an application of liquid *Azotobacter* significantly increased the plant height of marigold over the lignite based *Azotobacter* culture and uninoculated control.

30 Days after planting

The significantly superior plant height was recorded due to application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1= 16.25cm), followed by seed treatment alone with liquid biofertilizer (T_2= 14.95cm). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N.

(T_7) and 75% R.D.N. (T_{15}) recorded plant height of 14.14cm, 14.00cm respectively; which were at par with each other. The least plant height was recorded in uninoculated control (T_{16}=11.45cm).

60 Days after planting

Similar trend of the result was observed in respect of plant height at 60 DAP as that of 30 DAP. The significantly superior plant height was recorded due to application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1=26.65cm) over all other treatments, followed by seed treatment alone with liquid biofertilizer (T_2 =24.40cm). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N.(T_7) and 75% R.D.N. (T_{15}) recorded plant height of 23.00cm and 22.65cm respectively; which were at par with each other. The least plant height was recorded in uninoculated (T_{16}=16.17cm).

90 Days after planting

Application of liquid *Azotobacter* significantly increased the plant height at 90 DAP from 27.60cm to 41.05cm.The significantly superior plant height was recorded in treatment T_1= 41.05cm, followed by treatment T_2= 38.25cm. The treatments of foliar application of T_7 and T_{15} recorded plant height 35.90cm and 35.45cm respectively; which were at par with each other. The least plant height was recorded in T_{16}=27.60cm.

120 Days after planting

Similar trend was maintained at 120 DAP as that of 90 DAP. The significantly superior plant height was recorded in seed treated with Seed treatment + seedling root dipping treatment + soil application + foliar application liquid *Azotobacter* (T_1=50.50cm), followed by seed treatment alone with liquid biofertilizer (T_2=47.80cm). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N. (T_{15}) recorded plant height of 44.05cm and 43.05cm respectively; which were at par with each other. The least plant height was recorded in uninoculated control (T_{16}=34.30cm).

The results are in conformity with the observation recorded by Shivappa *et al.* (1976) and Khullar *et al.* (1978) who reported that there was significant increase in plant height of marigold due to *Azotobacter* inoculation. The results are also in agreement with reports by Reddy and Lakhdive (1982) in hybrid sorghum (CSH-5), Radhakrishnan and Mallikarjunaiah (1983) in vegetable crops, Sonawane and More (1983) in brinjal and Debnath (1997) in case of gladiolus. Dibut *et al.* (1993) reported the soil inoculation of dilute preparation of *Azotobacter chroococcum* immediately after sowing increased the plant height in onion.

Ghosh and Das (1998) reported that increase in plant height and number of shoots per plant when crop received both biofertilizers and growth regulators either in combination or singly. Similar findings were reported by Narayan *et al.* (2007) in tomato by treatment with 100% N + *Azotobacter*+ PSB and Singaravel *et al.* (2008) in okra by application of liquid biofertilizers.

Number of branches per plant

The observations recorded on average number of branches per plant as influenced by various treatments were recorded at 30, 60, 90 and 120 DAP and presented in (Table 6) which were found to be statistically significant.

30 Days after planting

The significant increase was noted in number of branches per plant due to application of different formulations of *Azotobacter* culture over lignite based *Azotobacter* culture and uninoculated control. The significantly higher number of branches per plant were observed with seed treatment + seedling root

Table 5
Effect of different *Azotobacter* formulations and their methods of application on plant height (cm) of marigold

Tr.no.	*Treatments*	*30 DAP*	*60 DAP*	*90 DAP*	*120 DAP*
T_1	(Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A)+100% R.D.N.	16.25	26.65	41.05	50.50
T_2	(Liq. *Azo*.S.T.) +100% R.D.N.	14.95	24.40	38.25	47.80
T_3	(Liq.*Azo*. S.R.D.T.)+100% R.D.N.	13.88	22.15	34.35	42.60
T_4	(Carr.*Azo*.S.T.+S.R.D.T.+S.A.) +100%R.D.N.	13.40	21.75	33.70	41.22
T_5	(Carr. *Azo*.S.T.) +100% R.D.N.	12.62	20.20	31.40	38.35
T_6	(Carr. *Azo*. S.R.D.T.)+100% R.D.N.	12.35	19.55	30.25	37.30
T_7	(Liq. *Azo*.F.A.) +100% R.D.N.	14.14	23.00	35.90	44.05
T_8	Control (No inoculation) +100% R.D.N.	11.70	16.45	27.87	35.26
T_9	(Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A) +75% R.D.N.	14.67	23.80	37.35	46.45
T_{10}	(Liq. *Azo*.S.T.) +75% R.D.N.	14.55	23.50	36.80	45.40
T_{11}	(Liq. *Azo*. S.R.D.T.) +75% R.D.N.	13.35	21.50	32.63	36.55
T_{12}	(Carr.*Azo*.S.T.+S.R.D.T.+S.A) +75% R.D.N.	12.80	20.85	32.47	39.52
T_{13}	(Carr. *Azo*.S.T.) +75% R.D.N.	12.15	18.70	29.74	36.73
T_{14}	(Carr. *Azo*. S.R.D.T.) +75% R.D.N.	12.00	18.50	28.75	36.01
T_{15}	(Liq. *Azo*.F.A.) +75% R.D.N.	14.00	22.65	35.45	43.05
T_{16}	Control (No inoculation)+75% R.D.N.	11.45	16.17	27.60	34.30
	S.E. (±)	0.30	0.54	0.39	0.50
	C.D (0.05)	0.91	1.60	1.18	1.48

dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1=16.13), followed by seed treatment alone with liquid biofertilizer (T_2= 15.34). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N.(T_{15}) recorded 13.50 and 13.03 numbers of branches per plant respectively; which were at par with each other. The least number of branches per plant was recorded in uninoculated control (T_{16}=8.33).

60 Days after planting

At 60 DAP the number of branches per plant increased over 30 DAP and ranged from 11.38 to19.90. The significantly superior number of branches was recorded due to application of seed treatment + seedling root dipping treatment + soil application + foliar application liquid *Azotobacter* (T_1 =19.90), followed by seed treatment alone with liquid biofertilizer (T_2 =18.25). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N. (T_{15}) recorded 16.53 and 16.04 numbers of branches per plant respectively; which were at par with each other. The least number of branches per plant was recorded in uninoculated control (T_{16}=11.38).

90 Days after planting

Similar trend was maintained at 90 DAP on that of 60 DAP. The significantly higher number of branches was recorded with application of T_1 (22.40) over all other treatments, followed by T_2 (21.15), The treatments of foliar application (T_7) and (T_{15})

recorded 18.93 and 18.28 number of branches per plant respectively; which were at par with each other. The least number of branches per plant was recorded in uninoculated control (T_{16}=13.78).

120 Days after planting

At 120 DAP the number of branches per plant increased over 90 DAP and ranged from 16.94 to 25.34. The significantly higher number of branches was recorded with application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1= 25.34) over all other treatments, followed by seed treatment alone with liquid biofertilizer (T_2 = 24.09). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N.(T_{15}) recorded 21.88 and 21.30 numbers of branches per plant respectively; which were at par with each other. The least number of branches per plant was recorded in uninoculated control (T_{16}=16.94).

The results are in conformity with that of Jackson *et al.* (1964) who found that inoculation with *Azotobacter* accelerate the stem and leaf growth of tomato. There was significant increase in leaf surface area and number of branches of chilli plant due to *Azotobacter* inoculation (Shivappa *et al.* 1976, Khullar *et al.* 1978) and Chandrikapure *et al.* (1999) in marigold. Sharma and Thakur (2001) reported that among individual treatments of biofertilizers, the application of *Azotobacter* result in significant improvement in growth parameters like height, number of branches, number of leaves etc. in tomato. Similar results were obtained by Ingle *et al.* (2008) which was at par with 75% N +*Azotobacter* + PSB.

Table 6
Effect of different *Azotobacter* formulations and their methods of application on number of branches per plant of marigold

Tr.no.	*Treatments*	*30 DAP*	*60 DAP*	*90 DAP*	*120 DAP*
T_1	(Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A.)+100% R.D.N.	16.13	19.90	22.40	25.34
T_2	(Liq. *Azo*.S.T.) +100% R.D.N.	15.34	18.25	21.15	24.09
T_3	(Liq. *Azo*. S.R.D.T.) +100% R.D.N.	12.22	15.17	17.57	20.52
T_4	(Carr.*Azo*.S.T.+S.R.D.T.+S.A.) +100%R.D.N.	12.07	15.07	17.47	20.42
T_5	(Carr. *Azo*.S.T.) +100% R.D.N.	10.80	13.82	16.22	19.17
T_6	(Carr. *Azo*. S.R.D.T.) +100% R.D.N.	10.54	13.54	15.94	18.73
T_7	(Liq. *Azo*.F.A.) +100% R.D.N.	13.50	16.53	18.93	21.88
T_8	Control. (No inoculation) +100% R.D.N.	8.87	12.06	14.46	17.41
T_9	(Liq.*Azo*.S.T.+S.R.D.T.+S.A.+F.A.) +75% R.D.N.	14.61	17.68	20.08	23.32
T_{10}	(Liq. *Azo*.S.T.) +75% R.D.N.	14.07	16.95	19.35	22.29
T_{11}	(Liq. *Azo*. S.R.D.T.) +75% R.D.N.	11.85	14.95	17.35	20.35
T_{12}	(Carr.*Azo*.S.T.+S.R.D.T.+S.A) +75% R.D.N.	11.50	14.50	16.90	19.79
T_{13}	(Carr. *Azo*.S.T.) +75% R.D.N.	9.77	12.71	15.11	18.05
T_{14}	(Carr. *Azo*. S.R.D.T.) +75% R.D.N.	9.35	12.40	14.80	17.75
T_{15}	(Liq. *Azo*.F.A.) +75% R.D.N.	13.03	16.04	18.28	21.30
T_{16}	Control. (No inoculation) +75%R.D.N.	8.33	11.38	13.78	16.94
	S.E. (±)	0.21	0.26	0.35	0.70
	C.D (0.05)	0.63	0.78	1.05	1.09

Number of flowers per plant

The data on number of flowers as influenced by different *Azotobacter* formulations with their different methods of application are recorded and presented in (Table 7).

There was significant increase in number of flowers per plant due to application of different *Azotobacter* formulations with their different methods of application. The maximum number of flowers was recorded with application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1= 40.60), followed by seed treatment alone with liquid biofertilizer (T_2=38.00). The treatments of foliar application of liquid *Azotobacter* of liquid *Azotobacter* with 100% R.D.N.(T_7) and 75% R.D.N. (T_{15}) recorded 34.75 and 34.50 numbers of flowers per plant; respectively which were at par with each other. The least number of flowers per plant was recorded in uninoculated control (T_{16}= 25.75).

Yield of marigold flowers

The data on fresh flower yield as influenced by different formulations of *Azotobacter* and their methods of application were recorded and presented in (Table 8) and graphically shown in Fig. 5. There was significant increase in yield of marigold flowers due to application of liquid *Azotobacter* and it varied between 83.12g plant^{-1} to 122.13g plant^{-1}.

The maximum flower yield of marigold was recorded with application of seed treatment + seedling root dipping treatment + soil application +

Table 7
Effect of different *Azotobacter* formulations and their Methods of application on number of flowers per plant

Tr. no.	*Treatments*	*Number of flowers per plant*	*Percent Increase/ decrease over control.*
T_1	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +100% R.D.N.	40.60	48.99
T_2	(Liq. *Azo.*S.T.) +100% R.D.N.	38.00	39.44
T_3	(Liq. *Azo.* S.R.D.T.) +100% R.D.N.	33.75	23.85
T_4	(Carr.*Azo.*S.T.+S.R.D.T.+S.A.)+100%R.D.N.	31.75	16.51
T_5	(Carr. *Azo.*S.T.) +100% R.D.N.	29.50	8.25
T_6	(Carr. *Azo.* S.R.D.T.) +100% R.D.N.	28.75	5.50
T_7	(Liq. *Azo.*F.A.) +100% R.D.N.	34.75	27.52
T_8	Control. (No inoculation) +100% R.D.N.	27.25	0.00
T_9	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +75% R.D.N.	36.75	42.71
T_{10}	(Liq. *Azo.*S.T.) +75% R.D.N.	36.00	39.80
T_{11}	(Liq. *Azo.* S.R.D.T.) +75% R.D.N.	31.00	20.38
T_{12}	(Carr.*Azo.*S.T.+S.R.D.T.+S.A)+75% R.D.N.	30.50	18.44
T_{13}	(Carr. *Azo.*S.T.) +75% R.D.N.	28.25	9.70
T_{14}	(Carr. *Azo.* S.R.D.T.) +75% R.D.N.	28.00	8.73
T_{15}	(Liq. *Azo.*F.A.) +75% R.D.N.	34.50	33.98
T_{16}	Control. (No inoculation) +75% R.D.N.	25.75	0.00
	S.E. (±)	0.34	-
	C.D (0.05)	1.01	-

foliar application of liquid *Azotobacter* (T_1 =122.13g plant^{-1}), followed by seed treatment alone with liquid biofertilizer (T_2 =116.67 g plant^{-1}). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N.(T_{15}) recorded 107.33 g plant^{-1} and 105.87 g plant^{-1} flower yield of marigold per plant respectively. The least flower yield of marigold per plant was recorded in uninoculated control (T_{16}=83.12g plant^{-1})

The results are similar to that of Shivappa *et al.* (1976) and Khullar (1977) who reported the increased yield of chilli due to *Azotobacter* inoculation. Similar results were also obtained by Khullar and Chahal (1977), Khullar *et al.* (1978) in carrot and Mandale (2003) in chilli. Ghany (1996) reported that seed inoculation with strains of *Azotobacter chroococcum, Azospirillum lipoferum* and its mixture have positive influence on yield of soybean. The results are similar to those of Panwar *et al.* (2000) in radish at 120 kg N /ha, Sharma (2002)in cabbage, Amer *et al.* (2003) in tomato and Talukdar and Jana (2009) in chilli.

Dry matter weight

Data in respect of dry matter weight of marigold plants at harvesting stage influenced by different *Azotobacter* formulations with their different methods of application are presented in (Table 9) which was found to be statistically significant. Different *Azotobacter* formulations with their methods of application increased the dry matter weight of marigold significantly over uninoculated control.

Table 8
Effect of different *Azotobacter* formulations and their methods of application on yield (g plant^{-1}) of marigold

Tr.no.	*Treatments*	*Weight of flowers per plant*	*Percent Increase/ decrease over control.*
T_1	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.)+100% R.D.N.	122.13	42.69
T_2	(Liq. *Azo.*S.T.) +100% R.D.N.	116.67	36.12
T_3	(Liq. *Azo.* S.R.D.T.) +100% R.D.N.	102.91	20.06
T_4	(Carr.*Azo.*S.T.+S.R.D.T.+S.A.)+100%R.D.N.	101.05	17.89
T_5	(Carr. *Azo.*S.T.) +100% R.D.N.	93.15	8.60
T_6	(Carr. *Azo.* S.R.D.T.) +100% R.D.N.	89.91	4.90
T_7	(Liq. *Azo.*F.A.) +100% R.D.N.	107.33	25.22
T_8	Control. (No inoculation) +100% R.D.N.	85.71	0.00
T_9	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +75% R.D.N.	112.06	34.81
T_{10}	(Liq. *Azo.*S.T.) +75% R.D.N.	109.91	32.23
T_{11}	(Liq. *Azo.* S.R.D.T.) +75% R.D.N.	97.08	16.79
T_{12}	(Carr.*Azo.*S.T.+S.R.D.T.+S.A)+75% R.D.N.	94.99	14.28
T_{13}	(Carr. *Azo.*S.T.) +75% R.D.N.	88.46	6.42
T_{14}	(Carr. *Azo.* S.R.D.T.) +75% R.D.N.	86.46	4.01
T_{15}	(Liq. *Azo.*F.A.) +75% R.D.N.	105.87	27.37
T_{16}	Control. (No inoculation) +75% R.D.N.	83.12	0.00
	S.E. (±)	0.45	-
	C.D (0.05)	1.34	-

Dry matter weight of shoot

Data on dry matter weight of shoot per plant presented in (Table 9) revealed that application of different *Azotobacter* formulations with their different methods of application improved the dry matter weight of shoot significantly over their respective lignite based *Azotobacter* culture and uninoculated control.

The significantly highest dry matter weight of shoot (32.20g plant^{-1}) was observed due to application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1), followed by seed treatment alone with liquid biofertilizer (T_2=30.81g plant^{-1}). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N.(T_7) and 75% R.D.N. (T_{15}) recorded 28.75 g plant^{-1} and 27.95 g plant^{-1} dry matter weight of shoot respectively; which were at par with each other. The least dry matter weight of shoot (g plant^{-1}) was recorded in uninoculated control (T_{16}= 23.72g plant^{-1}).

Dry matter weight of root

The data pertaining to dry matter weight of root per plant is presented in (Table 9). It was revealed that an application of different *Azotobacter* formulations with different methods of application significantly increased the dry matter weight of root over their respective uninoculated control.

The significantly highest dry matter weight of root (g plant^{-1}) was observed in treatment T_1=12.47g plant^{-1}, followed by T_2 =12.27g plant^{-1}. The treatments of foliar application T_7 and T_{15} recorded 11.24g plant^{-1} and 10.94g plant^{-1} dry matter weight of root respectively; which were at par with each other. The least dry matter weight of root (g plant^{-1}) was recorded in T_{16}= 8.78g plant^{-1}.

Total dry matter weight

From the shoot and root dry matter weight, total dry matter weight per plant was calculated and presented in (Table 9). It revealed that application of different *Azotobacter* formulations with different methods of application improved the total dry matter weight significantly over uninoculated control.

Significantly highest total dry matter weight (44.67g plant^{-1}) was observed due of application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* over all other treatments, followed by seed treatment alone with liquid biofertilizer (T_2 = 43.09g plant^{-1}). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and75% R.D.N. (T_{15}) recorded 39.99g plant^{-1} and 38.89g plant^{-1} total dry matter weight respectively; which were at par with each other. The least total dry matter weight (g plant^{-1}) was recorded in uninoculated control (T_{16}= 32.50g plant^{-1})

Similar observations were recorded by Mishustin and Naumova (1962) who found that seed inoculation with *Azotobacter* culture increased the development of shoots over the control. Similar results were recorded by Reddy and Lakhdive (1982) in hybrid sorghum (CSH-5), Sonawane and More (1983) in brinjal and Debnath (1997) in case of gladiolus. Dibut *et al.* (1983) reported that soil inoculation of dilute preparation of *Azotobacter chroococcum* 5lit /ha immediately after sowing increased the dry matter weight of onion.

Deokar and Sawant (2001) observed that biofertilizers significantly increased the dry matter yield of chilli. Similar findings were reported by Sajindranath *et al.* (2002) in okra by application of biofertilizer and growth regulators either singly or in combination. Chaudhari *et al.* (2008) noticed that treatment with liquid *Azotobacter* along with 60 kg N/ha remarkably improved the stem thickness, length of main inflorescence, number of spikelets and seed weight which resulted in increase in grain and dry matter yield of grain amarantha. Singaravel *et al.* (2008) reported that application of liquid biofertilizers significantly increased the growth characters like height, number of branches and dry matter of okra.

Table 9
Effect of different *Azotobacter* formulations and their methods of application on dry matter weight (g plant^{-1}) of marigold

Tr.no.	*Treatments*	*Shoot*	*Root*	*Total dry matter weight*
T_1	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.)+100% R.D.N.	32.20	12.47	44.67
T_2	(Liq. *Azo.*S.T.) +100% R.D.N.	30.81	12.27	43.09
T_3	(Liq. *Azo.* S.R.D.T.) +100% R.D.N.	27.04	10.86	37.90
T_4	(Carr.*Azo.*S.T.+S.R.D.T.+S.A.)+100%R.D.N.	26.46	10.60	37.07
T_5	(Carr. *Azo.*S.T.) +100% R.D.N.	25.09	9.48	34.57
T_6	(Carr. *Azo.* S.R.D.T.) +100% R.D.N.	24.75	9.20	33.96
T_7	(Liq. *Azo.*F.A.) +100% R.D.N.	28.75	11.24	39.99
T_8	Control (No inoculation) +100%R.D.N.	23.77	8.93	32.70
T_9	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +75% R.D.N.	29.92	11.57	41.46
T_{10}	(Liq. *Azo.*S.T.) +75% R.D.N.	29.05	11.51	40.56
T_{11}	(Liq. *Azo.* S.R.D.T.) +75% R.D.N.	26.01	10.49	36.50
T_{12}	(Carr.*Azo.*S.T.+S.R.D.T.+S.A)+75% R.D.N.	25.44	10.19	35.63
T_{13}	(Carr. *Azo.*S.T.) +75% R.D.N.	24.55	8.97	33.52
T_{14}	(Carr. *Azo.* S.R.D.T.) +75% R.D.N.	24.26	8.86	32.92
T_{15}	(Liq. *Azo.*F.A.) +75% R.D.N.	27.95	10.94	38.89
T_{16}	Control. (No inoculation) +75%R.D.N.	23.72	8.78	32.50
	S.E. (±)	0.41	0.23	0.86
	C.D (0.05)	1.23	0.68	2.57

Effect of different *Azotobacter* formulations and their methods of application on biological properties of soil during crop growth period.

Soil *Azotobacter* population

Soil *Azotobacter* population as influenced by application of different *Azotobacter* formulations with their different methods of application were recorded during crop growth period at different interval i.e. 45 DAP and 120 DAP.

Results regarding the *Azotobacter* population are presented in (Table 10). The increasing trend of *Azotobacter* population due to inoculation with different *Azotobacter* formulations was observed up to 45 DAP and it decreased at 120 DAP.

Ghany (1996) studied the influence of different biofertilizers types in wheat production and found the higher population of *Azospirillum* followed by *Azotobacter chroococcum*. Kanungo *et al.* (1997) examined the cultivars of rice with high N absorption efficiency harbored higher population of nitrogen fixing *Azotobacter spp.*, *Azospirillum spp.* and anaerobic bacteria. Debnath (1997) reported the presence of *Azotobacter* in rhizosphere of various flower crops grown in medium black soils. Further he observed maximum number of cells count from the rhizosphere of gladiolus followed by gerbera and rose.

Toukhy and Azeem (2000) reported that application of biofertilizers significantly increased the microbial activity of rhizosphere of barley.

Similar findings were obtained by Borollosy *et al.*(2001) in sorghum rhizosphere.

This has reflected in significant increase and growth parameters of marigold as compared to control. The effect was more pronounced in treatment T_1.

Initial *Azotobacter* population – 7.50 x 10^5 g^{-1} of soil 45 Days after planting

Inoculation of liquid *Azotobacter* to the marigold seeds increased the *Azotobacter* population over lignite based *Azotobacter* culture and uninoculated control and ranged from 10.00 to 20.70 c.f.u. x10^4 g^{-1} of soil.

Significantly highest population (20.70 x10^5 g^{-1} of soil) was recorded with application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1) over all other treatments, followed by seed treatment alone with liquid biofertilizer (T_2 =18.80 x10^5 g^{-1} of soil) which was on par with T_1. The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N. (T_{15}) recorded 10.88 x10^5 g^{-1} of soil and 10.62 x10^5 g^{-1} of soil *Azotobacter* population respectively; which were at par with each other. The least *Azotobacter* population was recorded in uninoculated control (T_{16}=10.00 x10^5 g^{-1} of soil).

120 Days after planting

Azotobacter population (Table 10) decreased from 45 DAP to 120 DAP and ranged from 6.87 to 18.67 x10^5 $g^{-1}$of soil. Significantly highest *Azotobacter* population was observed (18.67 x$10^5$$g^{-1}$of soil) with

Table 10
Soil *Azotobacter* population as influenced by different *Azotobacter* formulations and their methods of application (c.f.u. x 10^5 g^{-1} of soil)

Tr.no.	*Treatments*	*45 DAP*	*120 DAP*
T_1	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +100% R.D.N.	20.70	18.67
T_2	(Liq. *Azo.*S.T.) +100% R.D.N.	18.80	16.50
T_3	(Liq. *Azo.* S.R.D.T.) +100% R.D.N.	16.00	13.70
T_4	(Carr.*Azo.*S.T.+S.R.D.T.+S.A.)+100%R.D.N.	15.83	13.39
T_5	(Carr. *Azo.*S.T.) +100% R.D.N.	14.89	12.48
T_6	(Carr. *Azo.* S.R.D.T.) +100% R.D.N.	14.03	11.63
T_7	(Liq. *Azo.*F.A.) +100% R.D.N.	10.88	7.44
T_8	Control. (No inoculation) +100% R.D.N.	10.49	7.98
T_9	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +75% R.D.N.	18.60	16.13
T_{10}	(Liq. *Azo.*S.T.) +75% R.D.N.	17.20	14.80
T_{11}	(Liq. *Azo.* S.R.D.T.) +75% R.D.N.	15.45	12.80
T_{12}	(Carr.*Azo.*S.T.+S.R.D.T.+S.A)+75% R.D.N.	15.20	12.55
T_{13}	(Carr. *Azo.*S.T.) +75% R.D.N.	12.17	10.00
T_{14}	(Carr. *Azo.* S.R.D.T.) +75% R.D.N.	11.50	8.50
T_{15}	(Liq. *Azo.*F.A.) +75% R.D.N.	10.62	7.12
T_{16}	Control. (No inoculation) +75% R.D.N.	10.00	6.87
	S.E. (±)	0.11	0.29
	C.D (0.05)	0.33	0.88

application of T_1, followed by T_2 (16.50 x10^5 g^{-1} of soil),The treatments of foliar application (T_7) and (T_{15}) recorded 7.44 x10^5 g^{-1} of soil and 7.12 x10^5 g^{-1} of soil *Azotobacter* population respectively; which were at par with each other. The least *Azotobacter* population was recorded in uninoculated control (T_{16}=6.87 x10^5 g^{-1} of soil).

Effect of different *Azotobacter* formulations and their methods of application on chemical properties of soil

Nitrogen uptake by marigold crop

The uptake of nitrogen as influenced by different *Azotobacter* formulations with their different methods of application were studied and calculated by considering concentration of nutrients and dry matter production of marigold plant. The data in respect of nitrogen uptake by marigold plant is given in (Table 11).

It was observed from the data given in (Table 11) that the nitrogen uptake in the marigold crop was significantly increased due to different *Azotobacter* formulations over uninoculated control. Significantly highest nitrogen uptake (0.74g $plant^{-1}$) was observed with application of seed treatment + seedling root dipping treatment + soil application + foliar application of liquid *Azotobacter* (T_1) over all other treatments, followed by seed treatment alone with liquid biofertilizers (T_2= 0.71 g $plant^{-1}$). The treatments of foliar application of liquid *Azotobacter* with 100% R.D.N. (T_7) and 75% R.D.N (T_{15}) recorded 0.65 g $plant^{-1}$and 0.62 g $plant^{-1}$ nitrogen uptake respectively; which were at par with each other. The least nitrogen uptake was recorded in uninoculated control (T_{16}= 0.50 g $plant^{-1}$).

Table 11
Effect of different *Azotobacter* formulations and their methods of application on nitrogen uptake by marigold crop (g $plant^{-1}$)

Tr.no.	*Treatments*	*N conc. (%)*	*N uptake (g $plant^{-1}$)*
T_1	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +100% R.D.N.	1.67	0.74
T_2	(Liq. *Azo.*S.T.) +100% R.D.N.	1.65	0.71
T_3	(Liq. *Azo.* S.R.D.T.) +100% R.D.N.	1.61	0.60
T_4	(Carr.*Azo.*S.T.+S.R.D.T.+S.A.)+100%R.D.N.	1.62	0.59
T_5	(Carr. *Azo.*S.T.) +100% R.D.N.	1.60	0.55
T_6	(Carr. *Azo.* S.R.D.T.) +100% R.D.N.	1.57	0.53
T_7	(Liq. *Azo.*F.A.) +100% R.D.N.	1.63	0.65
T_8	Control. (No inoculation) +100% R.D.N.	1.56	0.51
T_9	(Liq.*Azo.*S.T.+S.R.D.T.+S.A.+F.A.) +75% R.D.N.	1.63	0.67
T_{10}	(Liq. *Azo.*S.T.) +75% R.D.N.	1.63	0.66
T_{11}	(Liq. *Azo.* S.R.D.T.) +75% R.D.N.	1.64	0.59
T_{12}	(Carr.*Azo.*S.T.+S.R.D.T.+S.A)+75% R.D.N.	1.59	0.56
T_{13}	(Carr. *Azo.*S.T.) +75% R.D.N.	1.57	0.52
T_{14}	(Carr. *Azo.* S.R.D.T.) +75% R.D.N.	1.59	0.52
T_{15}	(Liq. *Azo.*F.A.) +75% R.D.N.	1.60	0.62
T_{16}	Control. (No inoculation) +75% R.D.N.	1.54	0.50
	S.E. (±)	0.02	0.01
	C.D (0.05)	0.07	0.05

The results are similar to that of Patil (1990) who noted that the seed inoculation with *Azotobacter* alone and combination of three doses of fertilizers were beneficial to increase the uptake of nitrogen in sorghum (CSH-1).

Narula *et al.* (2000) studied an inoculation of 'P' responsive wheat varieties with soil isolates and strains of *Azotobacter chroococcum* and showed greater nitrogen uptake as compared with parent soil isolates. Shriram and Prasad (2001) reported that application of 80 kg N/ha along with biofertilizers and growth regulators increased the nutrient uptake of seed cotton. Praharaj *et al.* (2002) found that soaking of seed tubers in 1% urea + 1% $NaHCO_3$ with biofertilizers (*Azotobacter spp.*) increased the nitrogen uptake by tubers of potato.

Piao *et al.* (2005) reported that the application of non-symbiotic nitrogen fixing bacteria alone or with nitrogenous fertilizers significantly increased nitrogen uptake in rice. Singaravel *et al.* (2008) studied the effect of different liquid biofertilizers on the uptake of N by okra and he found increase in the nitrogen uptake.

CONCLUSION

It is concluded that the application nitrogen fixing liquid biofertilizers (*Azotobacter*) as a S.T.+S.R.D.T.+S.A.+F.A. with 100% or 75% R.D.N. was found significantly superior than its carrier based counter parts and improved the soil biochemical properties as well as fulfilled the nutrient requirement of marigold crop to a considerable extent.

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