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Effect of Bio-inoculants on Growth and Quality Seedling Production of Coriander (Coriandrum sativum L.) in Nursery Condition

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Abstract

Nursery experiments were conducted to select the suitable bio-inoculants and their combinations to improve the growth and biomass of Coriandrum sativum L. Coriander seeds were germinated and transplanted in earthen pot with a potting mixture of unsterilized substrate (Sand : Red soil : Farm Yard Manure)in the ratio of 2:1:1 and bio inoculants were inoculated individually and in combinations with Azospirillum, Phosphobacterium and Arbuscular Mycorrhizal fungi. Control plants also maintained for comparing growth performance. Root length, shoot length and biomass were recorded at 45 days after inoculation. Concentration of chlorophyll and protein in plant tissue were also estimated. Results showed that the bio-inoculants treated seedlings were increased the growth and biomass of coriander. Among all the treatments Azospirillum was found to be the most effective bioinoculant in increasing the growth, biomass and quality of coriander. It was recorded

INTRODUCTION

Organic farming is getting more popular these days, which accentuates shift from high volume production system to high value production system. For achieving this, management practices that conserve soil health, efficient nutrient supply systems that rely on organics instead of chemicals and integrated pest management play vital role. Among these, efficient, cheap and reliable nutrient supply system will ensure sustainability of the organic farming system. Bio fertilizers in combination with organic manures found as effective component in organic farming for reliable and cheap supply of nutrients. These combinations were ecologically safe and improve soil fertility by improving the soil physical, chemical, and biological condition. At present the pinch on fertilizer consumption is being felt more in India, since the country cannot afford to either import the required fertilizer at high cost and subsidize the sale to the farmers or build new fertilizer plants at formidable cost. Hence farmers are prepared to take to organic farming by using bio-inoculants.

Bio-inoculants are cost effective and eco-friendly natural inputs providing alternate source of plant nutrients, thus increasing farm income by providing extra yields and reducing input cost also. Bio-inoculants increase crop yield by 20-30%, replace chemical N & P by 25%, stimulate plant growth, activate soil biologically, restore natural fertility and provide protection against drought and some soil borne diseases. Bio-inoculants widely used in agriculture crops and Azospirillum is an important non-symbiotic associative, nitrogen fixing rhizosphere bacteria and fixes atmospheric nitrogen in soil [Krishnamoorthy, 2002]. It augments nitrogen fixation [Vijayakumari and Janardhanan, 2003]. Rice responds well to Azospirillum inoculation [Karthikeyan et al., 2003]. However, some evidence shows that this activity has been overlooked. Azospirillum lipoferum produced catechol-typesiderophores under iron-starved conditions that exhibited antimicrobial activity against various bacterial and fungal isolates [Shah et al.,1992]. These inoculants need more attention in view of their triple action of nitrogen fixation, bio-control and production of plant growth regulators. Phosphobacterium also produces auxin and gibberellin, which may have favorable effects on plant growth [Somani et al., 1990]. The stimulative effect of Phosphobacteria inoculation on plant growth in phosphorus deficient soil has been reported by Asea et al. [1988]. However, in many crops still it is at an experimental stage only.

Coriander generally known as “Dhania” (Coriandrum sativum L.) belongs to Apiaceae. It is cultivated in Rajasthan, Gujarat, Madhya Pradesh, Tamil Nadu, U.P., etc. and mainly used as a condiment for its medicinal properties as well as for culinary purposes as spice. Due to the multiple utility, there is a need to improve the sustainable growth and biomass of Coriander. Hence, the present study was undertaken to study the augmentation effect of different bio-inoculants on the growth, biomass and biochemical changes of Coriandrum sativum.

MATERIALS AND METHOD

Seeds

Coriander fruits were collected from Horticulture department, Agricultural College and Research Institute Madurai. Seeds were separated and graded and uniform size was used for raising seedlings. Seedlings were raised in a mixture of unsterilized sand: Red soil: Farm Yard Manure (2: 1: 1) in earthen pot in order to find out the suitable bio-inoculants and their combinations to achieve maximum overall growth and minimise the cost of seedling production of the following treatments were given seven days after germination.

Azospirillum and Phosphobacterium

Lignite based carrier culture of Azospirillum (Azospirillum brasilense) and Phosphobacterium (Bacillus megaterium var. phosphoticum) with a population load of 109 and 108 colony forming units / gram of peat soil respectively were obtained from the Agricultural College and Research Institute, Madurai, Tamil Nadu, India.

AM fungi

AM fungus (Glomus fasciculatum) was isolated and recorded as dominant species in the rhizosphere soil of Coriander. It was multiplied in pot culture in the sterilized mixture of sand : soil (1: 1 v/v) and maintained in the roots of Sorghum vulgare as the host plant. The inoculum contained extrametrical hyphae, chlamydospores and infected root segments were added in the root zones of each seedling.

Experimental design and Treatments

The experiment was conducted at the Department of Botany, Thiagarajar College, Madurai, Tamil Nadu, India. The experiment was set up in a completely randomized block design with 8 treatments and twenty four replicates.

Seedlings were raised in earthen pot with a potting mixture of unsterilized sand: red soil: farm yard manure (2: 1: 1). Seven days after germination, 10 grams of peat soil based culture of Azospirillum, Phosphobacterium and vermiculate based AM fungus were inoculated. All the plants were kept under identical nursery condition up to 45 days.

T1- Azospirillum (Azospirillum brasilense)

T2-Phosphobacterium (Bacillus megaterium var. phosphoticum)

T3- Arbuscular Mycorrhizal Fungi (AMF) (Glomus fasciculatum)

T4- Azospirillum + Phosphobacterium

T5- Azospirillum + AMF

T6- Phosphobacterium + AMF

T7- Azospirillum+ Phosphobacterium+ AMF

T8- Control (Sand : Red soil : Farm Yard Manure 2:1:1)

Seedling survival percentage was calculated using the following formula:

$$\text{Seedling survival percentage} = \frac{\text{Number of seedlings present in each treatment}}{\text{Total number of seedlings transplanted in each treatment}} \times 100$$

Harvesting and measurement

45 days after inoculation from each treatment, a total of 12 seedlings were randomly selected. Seedlings were carefully uprooted without disturbing the root system and washed in running tap water. Excess of water was wiped out by placing them between folds of blotting paper. Shoot length, root length and basal diameter were recorded. The seedlings were cut at collar region, dried separately at 70o C in paper bags in hot air oven and biomass estimation (root and shoot dry weight) was carried out using top pan electronic balance.

Assessment of Mycorrhizal infection

Mycorrhizal root infection was assessed following the procedure of Phillips and Hayman (1970). The root segments were placed in a 2.5 % aqueous solution of KOH (w/v) and boiled in a water bath at 90 o C for 15 minutes. The roots were rinsed in water and lightened in H_2O_2 (3 ml of 20 % NH_4OH in 30 ml H_2O_2) for 10-45 minutes. They were again thoroughly rinsed with water several times and acidified by soaking in 40 - 50 ml of 1 % HCl for 3 min. Acidified roots were stained in an acidic glycerol solution (500 ml glycerol, 450 ml H_2O, 50 ml 1 % HCl) containing 0.05% trypan blue. The trypan blue solution was poured off and the roots were de-stained in acidic glycerol at room temperature. The stained roots were mounted in a glass slide and percentage of infection was calculated.

Seedlings Quality Index

Seedlings Quality Index was calculated using the formula of Dickson et al. 1960

Total dry weight (g/ plant) =

Seedlings Quality Index (SQI) / Height (cm) + Shoot dry weight (g/plant) / Root collar diameter (mm) x Root dry weight (g/plant)

Estimation of Chlorophyll and Protein

Chlorophyll-a, chlorophyll-b and total chlorophyll content was estimated by the method of Yoshida et al. 1971 and total protein by Lowry et al. 1951.

Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test ($P< 0.05$) [Duncan,1955].

RESULTS

Seedling survival:

The highest (78%) germination and survival was recorded in C. sativum seedlings treated with Azospirillum (Table1), followed by Azospirillum+ Phosphobacterium (T4) treated seedlings and it was recorded 75% which was statistically on a par with seedlings treated with Azospirillum+ Phosphobacterium+ AMF (T7).

Shoot length, Root length and Basal diameter:

Significant differences in shoot length, root length and basal diameter were recorded in C. sativum seedlings inoculated with the different microbial inoculants compared to the uninoculated control (Table 1).

Shoot length:

From the analysis of growth data the individual inoculation of Azospirillum (T1) treated seedlings was found to be the most effective in increasing the growth. Among all the treatments, the individual inoculation with Azospirillum (T1) treated seedlings recorded maximum shoot length and it was recorded as 57.71% increase over the control. It was followed by combined inoculation of Azospirillum + Phosphobacterium (T4) with 45.00 % increase over control, 45 days after inoculation (Table 1).

Root length:

Significant differences in root length were recorded in C. sativum seedlings inoculated with different bio- inoculants compared to uninoculated control (Table1). Statistical analysis of growth data showed that the individual inoculation of Azospirillum (T1) was found to be the most effective in increasing the root length of seedlings.

Among all the treatments, the individual inoculation with Azospirillum (T1) showed maximum root length 15.82 cm (41.76% increase over the control). The combined inoculation of Azospirillum + Phosphobacterium+ AMF (T7) showed higher root length and was statistically on a par with Azospirillum + Phosphobacterium (T4) inoculated seedlings.

Shoot Biomass :

The data pertaining to dry matter accumulation of shoot, root and total biomass are presented in Table 2. Significant differences were observed among the treatments evaluated 45 days after inoculation. The highest biomass in the shoot was recorded in seedlings inoculated with Azospirillum (T1). It was statistically on a par with seedlings treated with Azospirillum + Phosphobacterium +AMF (T7). They registered 47% and 44% increase over control. (Table 2)

Root biomass :

Statistically highly significant difference was found in different type of microbial inoculation on root biomass of C. sativum seedlings. Inoculation of Azospirillum (T1) alone and in combination with other inoculants was found to significantly increase root biomass when compared to other treatments. Root biomass was highest in Azospirillum (T1) followed by Azospirillum + Phosphobacterium + AMF (T7) (Table 2).

Total biomass of seedling:

Seedling biomass was the highest in the Azospirillum (T1) treated seedlings and it was 48 % more than that of the control and it was statistically on a par with seedlings treated with Azospirillum + Phosphobacterium + AM (T7). In the dual inoculation seedlings inoculated in combination with Azospirillum recorded more biomass than the control (Table 2).

Seedling Quality Index:

Good quality seedlings were obtained from seedlings inoculated with Azospirillum (T1). Azospirillum + Phosphobacterium + AMF (T7) showed the next highest seedling quality index, followed by Azospirillum + Phosphobacterium+ (T5). Among the double inoculations Azospirillum + AMF (T5) showed the highest seedling quality index (Figure 1, Table 2).

Mycorrhizal infection:

Mycorrhizal infection was found only in seedlings inoculated with AM fungi and the combined inoculation of Azospirillum + Phosphobacterium+ AMF (T7) showed higher levels of infection followed by AMF (T3) inoculated seedlings (Table 2).

Total Chlorophyll content:

Total chlorophyll content was found to be maximum in the seedlings inoculated with Azospirillum (4.650 mg/g fresh weight of leaves) followed by Azospirillum + Phosphobacterium + AMF (3.49 mg/g fresh weight of leaves) (Table 3).

Protein content:

Among all the treatments, protein content in tissue of C. sativum seedlings was found to be maximum in the seedlings produced from single application of Azospirillum (0.048 mg/plant) and triple application of Azospirillum +Phosphobacterium + AMF (0.075mg/plant) followed by Phosphobacterium+ AMF (0.066mg/plant) treatment (Table 3).

DISCUSSION

Biologically active products, more appropriately called microbial inoculations, containing active strains of a selective microorganisms like Azospirillum, Phosphobacterium, Arbuscular mycorrhizae alone or in combination, help plant growth through different mechanisms, including biological nitrogen fixation and solubilization of insoluble phosphate fertilizer. In the present study, the height, diameter and dry matter and quality seedlings were higher in the C. sativum seedlings inoculated with bioinoculants. The increase of growth may be attributed to high accumulation of chlorophyll and protein in the plant tissue.

Nitrogen fixing bacteria of the genus Azospirillum have promoted plant growth of agronomically important field crops by 10 to 30% in the field experiment [Okon, 1985; Sumner,1990] crop yield increase in germination rate, plant height, leaf size [Saikia et al., 2001] enhanced minerals and water uptake, increased dry matter accumulation, root surface area, root diameter density and root hair [Okon and Kapulnik 1986]to support the earlier reports. In the present study, Azospirillum inoculated seedlings showed better growth and root biomass when compared to the control. Growth may be attributed due to increased root biomass [Wong and Sternberg,1979], and the production of gibberellins and cytokinin like substances [Tien et al.,1979] which promote the growth of the seedlings. The above results corroborate with earlier studies on quality seedling production of Casuarina equisetifolia [Rajendran.et al.,2003]; Moringa oleifera [Kasthuri Rengamani et al.,2006]; Acacia nilotica [Rajendran and Jayashree,2007] Delonix regia [Alagesaboopathi and Rajendran, 2009], Samanea saman[Mohan and Rajendran 2012].

Growth promoting effect of inoculation with Azospirillum and Phosphobacterium alone or dual inoculation with both non symbiotic biofertilizers was found in several tree species such as Casuarina [Rajendran.et al., 2003]; Casuarina trees treated in farm forestry [Rajendran and Devaraj, 2004] Feronia elephantum [Mohan and Rajendran 2014]. Similarly, In the present study Phosphobacterium inoculated seedlings produced better plant height, stem girth and total biomass. It may be due to inoculation of phosphate solubilizing microorganism Bacillus megaterium which has shown stable and consistent capacity to solubilize insoluble phosphorus and thus making it available to plants.

Phosphate plays a major role in the root development [Kuccy, 1987]. Stribley [1987] reported that P seems to be the most important nutrient involved, other nutrients such as N, P, K, Ca, and Mg are translocated along with AM hyphae. Inoculation with AM fungi is known to enhance plant growth by improving the mineral nutrient of the host plant [Abbott and Robson, 1982; Mohan and Rajendran 2012]. In the present study mycorrhizal infection in roots of seedlings were found only in the inoculated seedlings. It is also recorded that growth medium needs bioinoculants and AMF inoculated seedlings had improved growth and nutrient content especially P uptake in the present result corroborate with earlier reports by Verma and Jamaluddin, 1995.

In the present study dual inoculation of AMF with Phosphobacterium influence the growth and biomass of Coriander seedlings. It is relevant to mention here that Phosphobacterium by virtue of its capacity to multiply certain growth promoting substances like IAA and GA might induce the growth of C. sativum seedlings [Ramamoorthy, 1982; Gaur,1990]. Among all the treatments are combined inoculations of Azospirillum +

Phosphobacterium +AMF produced excellent growth, biomass and tissue nutrient concentration. The greater height, diameter and dry matter of the C. sativum seedlings due to co-inoculation of all the biofertilizers might strongly improve accumulation of nitrogen due to Azospirillum [Gunjal and Patil 1992; Mohan and Rajendran 2014]], more phosphorus uptake by Phosphobacterium [Kuccy ,1987] and VAM fungi [Young et al.,1988].

The total chlorophyll and soluble protein content was found to be maximum in the seedlings inoculated with Azospirillum. This increase is in agreement with other findings [MCArther and Kawlis, 1993] and was attributed [Singh et al.,1983] to the greater supply of nitrogen to growing tissues. Similarly increase in chlorophyll and soluble protein content was also recorded in shola species [Sekar et al., 1995] with inoculation of Azospirillum+ Phosphobacterium.

CONCLUSION

Increasing dry land farming and development technologies for arid lands with soil related constraints now acquire new importance and emerge as new frontiers for agricultural development. Increased agronomical production has to come through the adoption of better management technology. Long-term sustainability in agriculture is possible only through the use of low cost farm grown inputs, which work in harmony with nature. Bio-inoculants act as perpetually renewable inputs helping in better tree crop nutrient management and maintenance of soil health, better soil and water management leading to improved agricultural practices. It is inferred that under appropriate technology, the use of efficient microbial inoculants yield increased growth and biomass of Coriander seedlings. The present study clearly shows that the application of Azospirillum plays a significant role in increasing the growth response of C. sativum seedlings in a stipulated period, thereby producing good quality seedlings. These treated seedlings may perform better in nutrient impoverished soil too.

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