EFFECT OF CO - INOCULATION OF *Azotobacter* spp. AND *Trichoderma asperellum* WITH INORGANIC FERTILIZER ON GROWTH AND YIELD OF CARROT

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Phosphate solubilizing and Nitrogen fixing microorganisms based biofertilizers can be used to reduce the inorganic Nitrogen (N) and Phosphorous (P) based chemical fertilizer usage and increase the available nitrogen and phosphorus in soil. To accomplish this, Sri Lankan isolates of *Azotobacter spp*. and *Trichoderma asperellum* were combined with lower rates of chemical fertilizers to evaluate the growth and yield characters of carrot. This study was conducted at the Division of Soil Plant and Nutrition, Horticultural Crop Research and Development Institute (HORDI) Gannoruwa. Prior to the combination of both organisms, the compatibility test was done. The pot experiment was carried out in Completely Randomized Design (CRD), consisting six treatments and four replications. The Treatments were namely T1 (No Fertilizer), T2 (100% NPK - DOA Recommendation), T3 (50% NP + K), T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K), T5 (75% NP + K) and T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K). The results of the compatibility test revealed that the isolates of *T. asperellum* and *Azotobacter* spp. were completely compatible. According to the results of the pot experiment, the significantly ($P < 0.05$) longest shoot length, maximum number of leaves, highest root diameter, highest root length, maximum shoot fresh weight, root fresh and dry weights were observed from treatments T6 and T2 while maximum shoot dry weight was observed from treatments T4 and T6. Available phosphorus content in treated soil was higher in T2 and T6. These results demonstrated that combined use of Sri Lankan isolate *T. asperellum* and *Azotobacter* spp. with reduced levels of (25% and 50%) nitrogen and phosphorous based inorganic fertilizers could enhance growth and yield parameters of carrot equivalent or higher than those obtained by using full rates of Department of Agriculture Sri Lanka recommended inorganic fertilizers.

Keywords: *Azotobacter* spp, Carrot, Inorganic fertilizer, *Trichoderma asperellum*

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INTRODUCTION

Carrot (*Daucus carota*) is one of the most popular vegetables grown and consumed in numerous countries all over the world. Carrots contain an impressive selection of phytochemicals, including carotenoids, anthocyanins, and other phenolic compounds. This makes the vegetable a good source of dietary antioxidants, when included in the diet (Nagraj et al., 2020). Increasing use of chemical fertilizers in agriculture have become a major concern in Sri Lanka due to its threatening effects towards the various non-communicable diseases such as asthma, cancer, Chronic Kidney diseases. Chemical fertilizers may make a country self-sufficient in food production, but chemicals have an adverse impact both on the environment and living organisms. In addition, the chemical fertilizers are expensive (Sprent, 1990). Bio fertilizers used in conjunction with chemical fertilizers improve crop productivity and nutrient use efficiency. It is becoming difficult to meet the nutrient need of farming through chemical fertilizer alone due to its higher costs; the concept of integrated plant nutrient supply system is gaining ground (Roshni et al., 2019). Nitrogen (N) and phosphorus (P) are well-known fundamental nutrients needed by plant for their growth and development. Phosphate solubilizing microorganisms and Nitrogen fixing Microorganism based biofertilizers can be used to reduce the inorganic N and P based chemical fertilizer usage and increase the plant availability N and P requirements (Kale et.al., 1992). At present in Sri Lanka, the cost of production of carrot is increasing and the yield is gradually decreasing. Not only carrot the total agriculture production is endangered. The present study aims to test the possibility of combining the use of Sri Lankan isolates *Azotobacter* spp. and *T. asperellum* as bio fertilizers in combination with reduced rates of chemical fertilizers for the growth and yield characteristics of carrot. Therefore, introducing this type of research will be a great contribution to the Sri Lankan economy and the farming community.

METHODOLOGY

The study was conducted at the Division of Soil Plant and Nutrition, Horticultural Crop Research and Development Institute (HORDI) Gannoruwa. The experimental area is located at 7° 16' 36.6064" N, 80° 35' 44.1204" E. Previously isolated and identified fungal culture *T. asperellum* was obtained from the Division of Plant Pathology, HORDI. The gene sequence was deposited for this isolate in the National Centre for Biotechnology Information (NCBI) with the accession number MH727475. *T. asperellum* cultures was maintained in Potato dextrose Agar (PDA). *Azotobacter* spp. were isolated from soil samples collected from farms grown with Carrot by soil dilution spread plate technique using Ashby's Mannitol media. Morphological identification was done together with KOH test and Catalase test.

Compatibility test between *Azotobacter* spp. and *T. asperellum*

Dual culture plate method described by Siddiqui and Shaukat (2003) was applied to test the compatibility. Zone of inhibition (if any) was measured by applying formula suggested by Vincent in 1947. The test was performed in triplicates, and repeated to ensure the viability of the microorganisms.

Co-inoculation effect of *Azotobacter* spp. and *T. asperellum* added with inorganic fertilizer for growth and yield of carrot

Soil was collected from uncultivated land in Gannoruwa, Peradeniya for filling the pots and tested for pH, total phosphorous, available phosphorous, total nitrogen, available nitrogen, soil organic matter and electrical conductivity. 5 kg of sieved soil were filled to plastic pots and the water level was adjusted until it acquired field capacity. New Kuroda' variety of carrot was used as the experimental variety. Treatments were established based on different combinations of Nitrogen fertilizer as urea, Phosphorous fertilizer as Triple superphosphate (TSP), Potassium fertilizer as Muriate of Potash (MOP), *Azotobacter* spp. and *T. asperellum* inoculums. The treatment applied were: T1 is the control added with no fertilizers (No Fertilizer). T2 was added with MOP, Urea and TSP as recommended by the Department of Agriculture (DOA) without inoculum (100% NPK – DOA Recommendation) , T3 was added with MOP recommended by DOA together with Urea and TSP half the recommended level with no inoculum (50% NP + K),T4 was added with MOP as recommended by DOA together with the Urea and TSP half the recommended level also added with the inoculum - *Azotobacter* spp. and *T. asperellum* (50% NP + *Azotobacter* spp. + *T. asperellum* + K), T5 was added with TSP and Urea 75% of the DOA recommended and MOP as recommended by the DOA with no inoculum (75% NP + K) and T6 was added with TSP and Urea 75% of the DOA recommended and MOP as recommended by the DOA and added with the inoculum - *Azotobacter* spp. and *T. asperellum* (50% NP + *Azotobacter* spp. + *T. asperellum* + K). 50 ml of *Azotobacter* spp. and *T. asperellum* inoculums were added to the related treatments. All the agronomic practices were implemented according to the recommendation made by DOA. Data were collected as shoot length in centimetres, number of leaves, root length in centimetres, root diameter in centimetres, and fresh weight of shoot, fresh weight of roots, as well as dry weight of shoot and root were collected in grams. Available phosphorous content in the treated soil was determined. This experiment was carried out using a completely randomized block design and four replicates for each treatment.
All the statistical analysis done by using Analysis of Variance (ANOVA) and the significance of the difference among the treatment combinations means were estimated by the Duncan's Multiple Range Test (DMRT) at 0.05 level of probability using SPSS 26 statistical package.

RESULTS AND DISCUSSION

Isolation and Identification of *Azotobacter* spp.

In the present study, isolation of *Azotobacter* spp. was done in Ashby's Mannitol Agar media. Pal et al., (2017) reported that Ashby's Mannitol Agar Medium is Selective media for *Azotobacter* spp. their growth in the media confirms *Azotobacter*. Observed morphological characteristics of the colonies, KOH test and Catalase test results: Colony color – white creamy, Transparency – Translucent, Nature of colony – Glistening, Margin of colony – Entire, Colony surface –Smooth, Texture – Mucoid, KOH test – Negative and Catalase test – Positive. Colony morphology and biochemical test results of this investigation was similar with findings of Rueda et al., (2016).

Compatibility test between *Azotobacter* spp. and *T. asperellum*

Absence of inhibition zone around the disk indicated that *Azotobacter* spp. was compatible with *T. asperellum*. Therefore, *T. asperellum* and *Azotobacter* spp. isolates of this experiment exhibited 100% compatible with each other. The findings of Pavitra et al., (2022) reported that, *Azotobacter* was 95.56% compatibility with *T. asperellum*. After repeating the procedure with grown cultures taken from experiment plates, showed the *Azotobacter* spp. and *T. asperellum* grown well on media. This viability also indicated that both microorganisms are compatible to each other.

Co-inoculation effect of *Azotobacter* spp. and *T. asperellum* added with inorganic fertilizer for growth and yield of carrot

The soil pH of studied soil was 6.46 ± 0.04, thus soil was within the neutral range. Total P (mg kg^{-1}) was 1325.04 ± 2.51, Available P (mg kg^{-1}) was 4.54 ± 0.19 (low), Total N (%) was 0.15 ± 0.02 (moderate), Available N (%) was 0.02 ± 0.0006 (low), Organic matter content (%) was 1.3 ± 0.2 (low), Electrical conductivity was 0.08 ± 0.007 (low) thus it was non saline soil.

The influence of co-inoculation of *Azotobacter* spp. and *T. asperellum* with different rates of inorganic fertilizer levels on Carrot shoot length (cm), number of leaves, root diameter (cm) and root length (cm) were shown in Table 1. Significantly ($P < 0.05$) the highest shoot length was observed from the treatment T4 (i.e. 42.38 cm) and it was on par with other treatments tested, except T1. The maximum Number of leaves per plant was observed from the treatment T4 (i.e. 12.5) and it was identical to the treatments T6 (i.e. 12.25) and T2 (i.e. 11.25). The highest root diameter was observed from T4 (i.e. 9.38 cm) and T2 (i.e. 9.38 cm) which was statistically identical to the T3 (i.e. 7.13 cm), T5 (i.e. 6.88 cm) and T6 (i.e. 8.93 cm). The significantly maximum root length was observed from treatment T6 (i.e. 16.82 cm) and it was on par with treatments T2 (i.e.16.57 cm), T4 (i.e.16.47 cm) and T5 (i.e. 15.22 cm)

Table 1: Effect of different treatments on shoot length (cm), number of leaves, root diameter (cm) and root length (cm)

Treatments	Shoot length (cm)	No. of leaves	Root Diameter (cm)	Root Length (cm)
T1	33.75 ± 3.3^{c}	9.00 ± 0.8^{c}	6.25 ± 0.6^{b}	9.3 ± 0.6^{c}
T2	40.93 ± 4.5^{a}	11.25 ± 0.9^{ab}	9.38 ± 1.7^{a}	16.57 ± 1.4^{a}
T3	38.38 ± 4.5^{ab}	10.75 ± 1.3^{b}	7.13 ± 1.9^{ab}	13.82 ± 1.5^{b}
T4	42.38 ± 3.9^{a}	12.50 ± 0.6^{a}	9.38 ± 2.2^{a}	16.47 ± 1.1^{a}
T5	36.50 ± 1.7^{ab}	10.75 ± 0.9^{b}	6.88 ± 1.1^{ab}	15.22 ± 2.1^{ab}
T6	38.88 ± 2.9^{ab}	12.25 ± 0.5^{a}	8.93 ± 1.1^{ab}	16.82 ± 1.4^{a}

*The mean value ± standard deviation (n = 4). Values with the same letter do not differ significantly ($P < 0.05$).

The influence of difference treatments on shoot fresh and dry weights, root fresh and dry weights were shown in Table 2. The highest shoot fresh weight was observed from T4 (i.e. 26.73 g) which was statistically identical to the T6 (i.e. 24.84 g) and T2 (i.e. 22.88 g). The maximum shoot dry weight was observed from treatment T4 (i.e. 10.32 g) and it was identical to the T6 (i.e. 10.11 g). The significantly maximum root fresh and dry weights were observed from T6 (i.e. 33.65 g and i.e. 5.63 g) it was on par with T4 (i.e. 29.05 g and i.e. 4.57 g) and T2 (i.e. 25.52 g and 4.26 g).

Table 2: Effect of different treatments on shoot / root fresh and dry weights (g)

Treatments	Shoot Fresh weight (g)	Shoot Dry weight (g)	Root fresh weight (g)	Root Dry weight (g)
T1	11.79 ± 3.2^{c}	3.67 ± 1.4^{d}	16.28 ± 5.1^{c}	2.31 ± 1.0^{b}
T2	22.88 ± 2.2^{ab}	9.17 ± 0.71^{b}	25.52 ± 4.7^{ab}	4.26 ± 1.2^{ab}
T3	18.22 ± 5.5^{b}	6.66 ± 2.0^{c}	21.35 ± 8.3^{bc}	2.61 ± 1.3^{b}
T4	26.73 ± 2.3^{a}	10.32 ± 0.8^{a}	29.05 ± 6.8^{ab}	4.57 ± 1.2^{a}
T5	18.56 ± 2.3^{b}	7.61 ± 0.6^{bc}	20.69 ± 2.0^{bc}	2.67 ± 1.1^{b}
T6	24.84 ± 2.6^{a}	10.11 ± 0.9^{a}	33.65 ± 3.3^{a}	5.63 ± 2.4^{a}

*The mean value ± standard deviation (n = 4). Values with the same letter do not differ significantly ($P < 0.05$).

The available phosphorous in soil after harvesting was the highest in T6 (i.e. 27.49 mg kg^{-1}) which was statistically identical to treatment T2 (i.e. 25.52 mg kg^{-1}) were shown in Table 3. Moreover, T6 was higher than other treatments tested and the initially measured available phosphorous in soil (i.e. 4.54 mg kg^{-1}).

Table 3: Effect of different treatments on Available p in soil (mg kg^{-1}) after harvesting

Treatment Number	Available P in soil (mg kg^{-1})
T1	6.76 ± 0.3^{e}
T2	25.52 ± 1.2^{a}
T3	11.80 ± 2.7^{d}
T4	21.71 ± 0.9^{b}
T5	14.36 ± 2.3^{c}
T6	27.49 ± 1.4^{a}

*The mean value ± standard deviation (n = 4). Values with the same letter do not differ significantly ($P < 0.05$)

According to the obtained results for growth and yield parameters of Carrot, different treatments show statistically different results. All the treatments of combination of *Azotobacter* spp. + *T. asperellum* inoculated with reduced level of Urea and TSP carrot plants resulted in better plant growth and yield parameters as compared to their respective non-inoculated treatments, full rates of inorganic fertilizer applied treatment and no fertilizer treatment. This obtained results might be because of the co-inoculation of *Trichoderma* with *Azotobacter* spp. has achieved important synergistic effects for promoting plant growth increasing nutrient uptake, increasing plant height and growth parameters (Poveda and Eugui, 2022). Results observed in this investigation are supported by the findings of Yadav et al., (2013) and Razdari et al., (2021).

CONCLUSION / RECOMMENDATION

The results of the study demonstrated that co-inoculation of Sri Lankan isolate *T. asperellum* and *Azotobacter* spp. with reduced levels of (25% and 50%) nitrogen and phosphorous based inorganic fertilizers (Urea and TSP) could enhanced growth and yield parameters of carrot equivalent or higher than those obtained by using full rates of those inorganic fertilizers recommended by DOA Sri Lanka. Therefore, there is a possibility to use both isolates conjointly as bio fertilizer to replace 25% and 50% Urea and TSP recommendation level by DOA Sri Lanka.

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