The co-inoculation effect of *Azotobacter* spp. and *Trichoderma asperellum* with inorganic fertilizer on growth and yield of carrot

T.T.Gunasekara[1*], K.K.K.Nawarathna[2], A.G.B.Aruggoda[1], S.Kohombange[2]

[1]Department of Agricultural and Plantation Engineering, The Open University of Sri Lanka, Nawala, Nugeogoda, Sri Lanka.
[2]Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka.

***Correspondence Author** – T. T. Gunasekara , taniyagunasekaratg11@gmail.com , +94767958960

DOI: 10.29322/IJSRP.14.01.2024.p14522
https://dx.doi.org/10.29322/IJSRP.14.01.2024.p14522

Paper Received Date: 24th December 2023
Paper Acceptance Date: 18th January 2024
Paper Publication Date: 26th January 2024

Abstract- Fertilizers play a prominent role in increasing the productivity of crops in the country. Phosphate solubilizing and nitrogen fixing microorganisms based biofertilizers can be used to reduce the inorganic nitrogen and phosphorous based chemical fertilizer usage and increase the plant availability nitrogen and phosphorous requirement. This study was aimed to use Sri Lankan isolates *Azotobacter* spp. and *Trichoderma asperellum* combination with lower rates of chemical fertilizers to evaluate their efficiency as biofertilizers for growth and yield characters of carrot. Further, possibility of combination use of both microorganisms were assessed. 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. + *Trichoderma asperellum* + K), T5 (75% NP +K) and T6 (75% NP + *Azotobacter* spp. + *Trichoderma asperellum* + K). The results of compatibility test resulted with 100% compatibility of *Azotobacter* spp. and *Trichoderma asperellum* isolates respectively. According to the results of pot experiment, best results of growth and yield parameters were observed from treatments T4, T6 and T2. The results of the present study reveals that combined use of *Trichoderma asperellum* and *Azotobacter* spp. with reduced levels of (25% and 50%) nitrogen and phosphorous based inorganic fertilizers could enhance the growth and yield of carrot equivalent or higher than those obtained by using full rates of Department of Agriculture Sri Lanka recommended inorganic fertilizers.

Keywords- *Azotobacter* spp., Biofertilizer, Compatibility, Carrot, Inorganic fertilizer, *Trichoderma asperellum*

I. INTRODUCTION

Increasing use of chemical fertilizers in agriculture have become a major concern in Sri Lanka and all over the world due to its threaten 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. Bio fertilizers used in conjunction with chemical fertilizers improve crop productivity and nutrient use efficiency. 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 (Kale and Bano 1992). *Trichoderma* is one of the most important fungi in agriculture. It has demonstrated many capabilities to be used as biofertilizers as well as biofungicides. *Trichoderma* produces plant growth hormones and volatile compounds, contributes to solubilizing phosphates that are unavailable to the crop and takes part in promoting the uptake of macro and micro nutrients needed by the crop (Kubheka and Ziena 2022). These capabilities makes it used as biofertlizer. *Azotobacter* spp. (Gram-negative prokaryote) are considered to improve the plant health. Various mechanisms are implicated behind improved plant health and growth in *Azotobacter* spp. Key characteristics of *Azotobacter* spp. are acceleration of phytohormone like Indole-3-Acetic Acid production, obviation of various stressors, nitrogen fixation, pesticides and oil globules degradation, heavy metals metabolization (Sumbul et al. 2020). The co-inoculation of *Trichoderma* with different Plant growth promoting rhizobacteria (PGPR) has achieved important synergistic effects for promoting plant growth increasing nutrient uptake, and/or increasing yield in different crops (Poveda and Eugui 2022).

Carrot (*Daucus carota*) is one of the most popular vegetables grown and consumed in numerous countries all over the world. At present in Sri Lanka and all over the world, the cost of production of carrots has increased and yield has gradually decreased. Not only carrot total agriculture production become endangered. With the increased cost of inorganic fertilizers, the application of the recommended dose is difficult to afford by small and marginal farmers. The application of biofertilizers containing a combination of beneficial microorganisms in a formulation with a reduced amount of chemical fertilizer was believed to be more effective compared with the stand-alone application of biofertilizers or chemicals (Zainuddin et al. 2022). Therefore, the present study aim to test the possibility of combining use of isolates *Azotobacter* spp. and *Trichoderma asperellum* in combination with lower rates of inorganic fertilizers for the growth and yield characteristics of carrot.

II. MATERIALS AND METHODOLOGY

The study was conducted at the Division of Soil Plant and Nutrition, Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Sri Lanka located at N 7° 16' 36.6064", E 80° 35' 44.1204". The soil type belongs to the Ultisoils and Great soil group was Reddish Brown Podsolic.

Isolation, Identification and Purification of *Azotobacter* spp.

Azotobacter spp. were isolated from soil samples collected from farms growing carrot by soil dilution spread plate technique using Ashby's Mannitol medium consisting of Mannitol 20.0 g, Dipotassium Phosphate 0.20 g, Magnesium Sulphate 0.20 g, Sodium Chloride 0.20 g, Potassium Sulphate 0.10g, Calcium Carbonate 5.00 g, Agar 15.00 g, pH 7.4 ± 0.2 for 1000 mL distilled water (Rao 1977). After incubation for 3 - 4 days *Azotobacter* spp. colonies grown in Ashby media were introduced to Ashby mannitol broth (Mannitol 20.0 g, Dipotassium phosphate 0.20 g, Magnesium sulphate 0.20 g, Sodium chloride 0.20 g, Potassium sulphate 0.10 g, Calcium carbonate 5.00 g, pH 7.4 ± 0.2 for 1000 mL distilled water. Samples of the culture were transferred with a loop sterilized Ashby's mannitol broth. This process was done several times to ensure growth of *Azotobacter* spp. in media. Identification of the isolate was done using morphology together with the KOH and catalase test.

Preparing *Azotobacter* spp. Stock culture for preservation

To preserve *Azotobacter* spp. 30% (v/v) glycerol solution was prepared, by mixing 30 mL of glycerol with 70 mL of distilled water. The solution was sterilized in an autoclave at 121°C, 15 psi for 20 min. Sterilized 0.5 mL of the 30% (v/v) glycerol solution was transferred to the sterile Eppendorf tube followed by transferring 1.5 mL of *Azotobacter* spp. grown in Ashby's Mannitol broth, mix well store at 4 ^{0}C. Same procedure was done using *Azotobacter* spp. colonies grown in Ashby's Mannitol Agar media.

T. asperellum culture selection

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 Center for Biotechnology Information (NCBI) with the accession number MH727475.

Preparing *T. asperellum* Stock cultures for preservation

T. asperellum cultures were preserved in sterilized 20 % (v/v) and 50% (v/v) glycerol solution, by mixing 20 mL of glycerol with 80 mL of distilled water and 50 mL of glycerol with 50 mL of distilled water respectively. Prepared solution was sterilized by using autoclave at 121 ^{0}C temperature, 15 psi for 20 minutes. The solution was sterilized in an autoclave at 121 °C, at 15 psi, for 20 min. *T. asperellum* grown in Potato Dextrose Broth (PDB) and Potato Dextrose Agar (PDA) were used for the preservation. Under aseptic conditions 1 mL of the 20% (v/v) glycerol solution was transferred to a sterile Eppendorf tube and the broth culture cut into small pieces and transferred to an Eppendorf tube using a sterilized loop, mixed well, and stored at 4°C. Moreover, Two-mm of mycelia of *T. asperellum* grown in PDA medium was transferred using a sterile loop and to 1 mL of 20% (v/v) glycerol solution. The solution was mixed well, and stored at 4°C. The same procedure was applied for 50% (v/v) glycerol solution preparation.

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

The dual culture plate method described by Siddiqui and Shaukat (2003) was applied to test the compatibility of the organisms. A zone of inhibition (if any) was measured using the formula of Vincent (1947). The test was performed in triplicate, and repeated to ensure viability of the microorganisms.

Azotobacter spp. and *T. asperellum* inoculum preparation

Identified colonies were transferred with a loop from Ashby's Mannitol agar medium and to Ashby's Mannitol broth under aseptic conditions and incubated at room temperature in a shaker (Barnstead-Lab line Max Q 3000 Platform Shaker®, USA) at 120 rpm for 7 days to inoculum preparation. *T. asperellum* was cultured in PDA medium consisting of infusion from potatoes – 200 g, Dextrose – 20 g, Agar – 15 g and pH after sterilization (at 25 °C) 5.6 ± 0.2 for 1000 mL distilled water. At 7 days after incubation, a loop full of spores and mycelium of *T. asperellum* were aseptically transferred to PDB, mixed well and incubated at room temperature in a shaker (Barnstead-Lab line Max Q 3000 Platform Shaker®, USA) at 120 rpm for 7 days to inoculum preparation. The spores count of *T. asperellum* inoculum was measured by using the Haemocytometer (Model - Hawksle®, United Kingdom) and *Azotobacter* spp. count in the inoculum measured by using spread plate method followed by serial dilution.

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

Soil was collected from uncultivated land in HORDI, Gannoruwa, and used to fill the pots. The soil was tested for pH, total phosphorous, available phosphorous, total nitrogen, available nitrogen, soil organic matter and electrical conductivity followed by methods described in Table 1.

Table 1 Methods used for soil analysis

Chemical property	Method
Soil pH	Extraction of 1:1 soil and water and determined by pH meter- Eutech® pH meter, Singapore (Mk and Davey 1988)
Total P	Aqua regia digestion method, Ascorbic acid color development method (Gasparatos and Haidouti 2001).
Available P	Extraction by Olsens's method (Olsen et al. 1954), Color developed by the method developed by Murphy and Relay (1962) and absorbance was determined by Spectrophotometer (Jenway 6305®, United Kingdom).
Total N	Devarda's Alloy method (Liao 1981).
Available N	Alkaline permanganate method (Subbiah and Asija 1856).
Soil organic matter	Loss on Ignition method using muffle furnace (Ben-Dor and Banin 1989).
Electrical Conductivity	Extraction of 1:5 soil and water and determined by Conductivity meter – Eutech, Singapore (Corwin et al. 2003).

Four-kg of sieved soil were placed in plastic pots and water level adjusted to 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 treatments applied were: T1 the control with no fertilizers (No Fertilizer). T2 was added with MOP, Urea and TSP as recommended by the Department of Agriculture (DOA) Sri Lanka without inoculum (100% NPK – DOA Recommendation), T3 was added with MOP recommended by DOA together with half the recommended level Urea and TSP 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 and 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 diluted and added to the related treatments during fertilizer application days. Further sterilized inoculums were applied to the related treatments. All the agronomic practices were implemented according to the recommendation made by DOA. Data were collected as shoot height (cm), number of leaves, root length (cm), root diameter (cm), and fresh weight of shoot (g), fresh weight of roots (g), as well as dry weight of shoot and root (g) were collected. Available phosphorous (mg kg^{-1}) 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.

III. RESULTS

Isolation and Identification of *Azotobacter* spp.

Observed morphological characteristics of the colonies, KOH test and Catalase test results: Colony colour – white creamy, Transparency – Translucent, Nature of colony – Glistening, Margin of colony – Entire, Colony surface – Smooth, Texture – Mucoid, KOH test – Negative and Catalase test – Positive.

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

Inhibition zone was absence around the disk. After repeating the procedure with grown cultures taken from experiment plates, showed the *Azotobacter* spp. and *T. asperellum* grown well on media.

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

Initial soil characteristics

The soil pH of studied soil was 6.46 ± 0.04, total P (mg kg^{-1}) is 1325.04 ± 2.51, available P (mg kg^{-1}) was 4.54 ± 0.19, total N (%) was 0.15 ± 0.02 , available N (%) was 0.02 ± 0.0006, organic matter content (%) was 1.3 ± 0.2 and electrical conductivity was 0.08 ± 0.007.

Growth parameters

Improvement in growth characters is considered to be a pre-requisite to increase the yield of crops. In this present study found that different treatments were found to have significant effect on growth characters such as shoot height (cm), number of leaves per plant, shoot fresh and dry weights (g) shown in Table 2.

Table 2 Effect of different treatments on growth characters of Carrot

Treatment Number	Shoot height (cm)	No. of leaves	Shoot Fresh weight (g)	Shoot Dry weight (g)
T1	33.75 ± 3.3^{b}	9.00 ± 0.8^{c}	11.79 ± 3.2^{c}	3.67 ± 1.4^{d}
T2	40.93 ± 4.5^{a}	11.25 ± 0.9^{ab}	22.88 ± 2.2^{ab}	9.17 ± 0.71^{ab}
T3	38.38 ± 4.5^{ab}	10.75 ± 1.3^{b}	18.22 ± 5.5^{b}	6.66 ± 2.0^{c}
T4	42.38 ± 3.9^{a}	12.50 ± 0.6^{a}	26.73 ± 2.3^{a}	10.32 ± 0.8^{a}
T5	36.50 ± 1.7^{ab}	10.75 ± 0.9^{b}	18.56 ± 2.3^{b}	7.61 ± 0.6^{bc}
T6	38.88 ± 2.9^{ab}	12.25 ± 0.5^{a}	24.84 ± 2.6^{a}	10.11 ± 0.9^{a}

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

Significantly ($P < 0.05$) the highest shoot height was observed from the treatment T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K) and it was statistically on par with treatments between T2 (100% NPK – DOA Recommendation), T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K), T5 (75% NP + K), T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) and T3 (50% NP + K). Maximum Number of leaves per plant were observed from the treatment T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K) and There were no significant difference between T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K), T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) and T2 (100% NPK – DOA Recommendation). Further, significant variation was found in respect of weight of fresh shoot. The maximum shoot fresh weight was observed from treatment T4 (50% NP + A*zotobacter* spp. + *T. asperellum* + K) and it was on par with treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) and T2 (100% NPK – DOA Recommendation). The significantly maximum shoot dry weight of the plant was recorded in treatment T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K) and it was par with treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K).

Yield Parameters

The present study investigated that the different treatments had significant effects on yield characters such as root length (cm), root diameter (cm), root fresh and dry weights (g) shown in Table 3.

Table 3 Effect of different treatments on yield characters of Carrot

Treatment Number	Root Diameter (cm)	Root Length (cm)	Root fresh weight (g)	Root Dry weight (g)
T1	6.25 ± 0.6^{b}	9.3 ± 0.6^{c}	16.28 ± 5.1^{c}	2.31 ± 1.0^{b}
T2	9.38 ± 1.7^{a}	16.57 ± 1.4^{a}	25.52 ± 4.7^{ab}	4.26 ± 1.2^{ab}
T3	7.13 ± 1.9^{ab}	13.75 ± 1.5^{b}	21.35 ± 8.3^{bc}	2.61 ± 1.3^{b}
T4	9.38 ± 2.2^{a}	16.47 ± 1.1^{a}	29.05 ± 6.8^{ab}	4.57 ± 1.2^{ab}
T5	6.88 ± 1.1^{ab}	15.22 ± 2.1^{ab}	20.69 ± 2.1^{bc}	2.67 ± 1.1^{b}
T6	8.93 ± 1.1^{ab}	16.83 ± 1.1^{a}	33.65 ± 3.4^{a}	5.63 ± 2.4^{a}

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

Root diameter in carrot plants varied significantly different among treatments applied ($P < 0.05$). Maximum root diameter was recorded under T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K) which was at par with T2 (100% NPK - DOA Recommendation), T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K), T3 (50% NP + K) and T5 (75% NP + K). Significant maximum root length was observed from treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) whilst there was no significant differences between T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K), T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) and T2 (100% NPK - DOA Recommendation). Further, T5 (75% NP+ K) did not show any significant difference ($P < 0.05$) comparing it with its respective inoculated treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K). The maximum fresh weight of root was recorded under treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) and it was on par with treatments T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K) and T2 (100% NPK - DOA Recommendation). Significantly, the maximum dry weight of root was recorded under treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) which was on par with T4 (50% NP + *Azotobacter* spp. + *T. asperellum* + K) and T2 (100% NPK - DOA Recommendation). Moreover, see Fig. 1.

Fig. 1 Co – inoculation effect of *Trichoderma asperellum* and *Azotobacter* spp. with Inorganic Fertilizer on Carrot - After harvesting

Available phosphorous in soil (mg kg^{-1}) after harvesting

Significant differences observed among the treatments with respect to available phosphorus in soil after harvesting were shown in Table 4. The maximum available phosphorous in soil was recorded in treatment T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) which was statistically identically to T2 (100% NPK – DOA Recommendation). Moreover, T6 (75% NP + *Azotobacter* spp. + *T. asperellum* + K) was higher than other treatments and the initially measured available phosphorous in soil.

Table 4 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}

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

IV. 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* spp. Further, colony morphology and biochemical test results of this investigation were similar to the studies of Hindersah et al. (2021) and Rueda et al. (2016).

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

The absence of inhibition zone around the disk indicated that *Azotobacter* spp. was compatible with *T. asperellum*. Therefore, both microbes exhibited 100% compatible with each other. The findings of Pavitra et al. (2022) reported that *Azotobacter* was 95.56% compatible with *T. asperellum*. Moreover, he stated both microorganisms are mutual in nature hence they were compatible with each other. After repeating the procedure with grown cultures taken from experiment plates it was shown that both microbes grew well on media. This viability also indicated that both microorganisms are compatible with each other.

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

Initial soil characteristics

Soil pH of studied soil was within the neutral range. Soil available P was low. Total N was moderately level. Available N percentage was low. Organic matter content was low. Electrical conductivity was low thus it was non saline soil.

Growth characters

Application of *T. asperellum* and *Azotobacter* spp. with cut down of 50% TSP and Urea treatment (T4) was exhibited the higher shoot height. Significantly it was no difference to treatments T3, T2, T6 and T5. But quantitatively it was different. This 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). Therefore, this can be the reason for the results of highest number of leaves for inoculated treatments observed in this investigation. Plants inoculated with bio-stimulants favor grass growth in reduced doses of mineral fertilizers. The results of shoot fresh weight may be due to the significant increase in root values may be related to increases in the availability of minerals especially N and P due to N fixation by *Azotobacter* spp. and P solubilize by *T. asperellum* that may lead to an increase in photosynthesizing surface. Thus, an increase in the accumulation of simple sugars and starch in roots occurred and resulted in the enhancement of roots and shoots. Result of this was the enhancement of shoot weight. Chatterjee et al. (2012) reported that co-inoculation of phosphate solubilizing and nitrogen fixing microorganisms increased the biomass of roots and shoots compared with a single inoculation. Moreover, the results of shoot fresh and dry weights may be due to the higher shoot length and more number of leaves of these treatments. Further, co-inoculation with *Trichoderma* and *Azotobacter* cell suspensions led to a synergistic increase in the plant growth promotion capacity of both microorganisms in different crops, due to a synergistic increase in IAA (Indole Acetic Acid) production (Battini et al. 2015; Syafiq et al. 2021).

Yield Characters

According to the results obtained in root diameter, a half dose of Urea and TSP with *T. asperellum* and *Azotobacter* spp. inoculated treatment, which mean T4 was identical to the T2, T3, T5 and T6 treatments. Deficiency of nutrients may be due to the results of the lowest diameter in T1. Palad and Aminah (2021) reported that *Trichoderma* species are able to stimulate growth by affecting the balance of hormones such as IAA, gibberellic acid and ethylene, which creates a favorable environment for nutrient uptake to form optimal plant growth and development through treatment and further stated that increase in accumulation of simple sugars and starch in roots occurred and resulted in enhancement of roots. Wani et al. (2013) reported that *Azotobacter* also acts as plant growth enhancing agents through the production of phytohormones (auxins, cytokinins and gibberylins) which were the main substances that can increase and control plant growth. The results of root length may be due to the fungal solubilization of phosphates increase soil P and Nitrogen fixation increase N results in increase of N and P up to the department recommendation of P and N that was made use the for root growth. *T. asperellum* and *Azotobacter* interaction were linked to a higher nutrient uptake, mainly N and P. There was a significance difference between inoculated treatments and their non-inoculated respective treatments and control treatment related to the root fresh and dry weights. T2 treatment (100% NPK – DOA Rec.) was not significantly different from inoculated treatments. The results of root fresh and dry weights were might be due to the higher root length and root diameter. Moreover, application of inorganic fertilizer with biofertilizer resulted with the increase of the root fresh and dry weights. This significant increase in root values may be related to increases in the availability of minerals especially N due to N fixation that may lead to an increase photosynthesizing surface. Badar (2012) reported that *Trichoderma* – bacteria application together increases plant biomass, nutrient uptake, and crop yield in *Vigna mungo*. This linkage caused higher content of chlorophyll, carbohydrates, and foliar proteins. Synergistic effect increase not only on plant biomass but also on N and P uptake. Karuppiah et al. (2019) stated that the production of PGP compounds by both microorganisms led to a synergistic increase in root and shoot length, as well as fresh plant mass. Further, the results of growth and yield characters were completely or somewhat conformity with findings of in Velmourougane et al. (2019) in Wheat and Cotton, Balbande et al. (2023) in radish, Vithwell and Kanaujia (2014) and Baba et al. (2018) in tomato.

Available phosphorous in soil (mg kg-1) after harvesting

According to the results, *T. asperellum* solubilized P up to more than NPK treatment level. Singh et al. 2014 stated that application of phosphate-solubilizing microorganisms to the soil increased plant growth by solubilization of insoluble phosphates and subsequent increase of nitrogen fixation. Therefore, inoculation of this *Azotobacter* spp. and *T. asperellum* combination with NPK fertilizer might be increased availability and uptake of phosphates. These results were accordance with findings of Püschel et al. 2017.

CONCLUSION

The results of the study demonstrated that *T. asperellum* and *Azotobacte*r spp. isolates used in this experiment were exhibited 100% compatible with each other. Further, co-inoculation of isolate *T. asperellum* and *Azotobacter* spp. with reduced levels of (25% and 50%) nitrogen and phosphorous based inorganic fertilizers (Urea and TSP) could enhance 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 fertilizers to replace 25% and 50% Urea and TSP inorganic fertilizer requirement.

ACKNOWLEDGMENT

This work was supported by the National Agricultural Research Plan Project (NARP) under Grant 285-02-02-15-2507-11 and Ministry of Agriculture Research Plan Project under titled “Development of Environmentally sound sustainable plant protection and nutrient management technologies in vegetable to minimum use of agrochemicals”. Moreover, authors are grateful to the Dr. R. G. A. S. Rajapaksha for providing the *T. asperellum* isolate for this study.

COMPLIANCE WITH ETHICAL STANDARDS

a) Conflicts of interest

The authors declare that they have no conflict of interest.

b) Research involving Human Participants and/or Animals

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, [T. T. Gunasekara], upon reasonable request.

REFERENCES

Ahmad Baba Z, Tahir S, Shafiq Wani F, Hamid Burhan, Nazir M, Hamid Basharat (2018) Impact of *Azotobacter* and inorganic fertilizers on yield attributes of tomato. Int J Curr Microbiol Appl Sci. 7(2):3803–3809. doi:10.20546/ijcmas.2018.702.450. http://dx.doi.org/10.20546/ijcmas.2018.702.450.

Badar R (2012) Comparative effect of *Trichoderma hamatum* and host-specific Rhizobium species on growth of Vigna mungo. J Appl Pharm Sci.:128–132. doi:10.7324/japs.2012.2409. http://dx.doi.org/10.7324/japs.2012.2409.

Balbande V, Tembhre D, Sengar C, Bhandari J (2023) Effect of integrated nutrient management on growth, yield and quality of radish (*Raphanus sativus* L.) cv MAHY 22. Pharma Innov J. 12(3):1103–1107.

Battini K, Kasa P, Modugapalem H (2015) Isolation, screening, and molecular characterization of plant growth promoting rhizobacteria isolates of *Azotobacter* and *Trichoderma* and their beneficial activities. J Nat Sci Biol Med. 6(2):360. doi:10.4103/0976-9668.160006. http://dx.doi.org/10.4103/0976-9668.160006.

Ben-Dor E, Banin A (1989) Determination of organic matter content in arid-zone soils using a simple "loss-on-ignition" method. Commun Soil Sci Plant Anal. 20(15–16):1675–1695. doi:10.1080/00103628909368175. http://dx.doi.org/10.1080/00103628909368175.

Chatterjee S, Sau GB, Sinha S, Mukherjee SK (2012) Effect of co-inoculation of plant growth-promoting rhizobacteria on the growth of amaranth plants. Arch Acker Pflanzenbau Bodenkd. 58(12):1387–1397. doi:10.1080/03650340.2011.595002. http://dx.doi.org/10.1080/03650340.2011.595002.

Corwin DL, Yemoto K (2019) Measurement of soil salinity: Electrical conductivity and total dissolved solids. Soil Sci Soc Am J. 83(1):1–2. doi:10.2136/sssaj2018.06.0221. http://dx.doi.org/10.2136/sssaj2018.06.0221.

Gasparatos D, Haidouti C (2001) A comparison of wet oxidation methods for determination of total phosphorus in soils. J Plant Nutr Soil Sci. 164(4):435. doi:10.1002/1522-2624(200108)164:4<435::aid-jpln435>3.0.co;2-5. http://dx.doi.org/10.1002/1522-2624(200108)164:4<435::aid-jpln435>3.0.co;2-5.

Hindersah R, Firmansyah Y, Kurniati N (2021) Soil properties of agricultural area in karst terrain of Parakan, Pangandaran, West Java, Indonesia. J Degrad Min Lands Manag. 8(3):2809–2814. doi:10.15243/jdmlm.2021.083.2809. http://dx.doi.org/10.15243/jdmlm.2021.083.2809.

Kale RDBC, Bano DJ (1992) Influence of vermicompost application on the available micronutrients and selected microbial population. Soil Biology and Biochemistry. 24(12):1317–1320.

Karuppiah V, Sun J, Li T, Vallikkannu M, Chen J (2019) Co-cultivation of *Trichoderma asperellum* GDFS1009 and Bacillus amyloliquefaciens 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity. Front Microbiol. 10. doi:10.3389/fmicb.2019.01068. http://dx.doi.org/10.3389/fmicb.2019.01068.

Liao CFH (1981) Devarda's Alloy Method for Total Nitrogen Determination1. Soil Science Society of America Journal. 45(5). doi:10.2136/sssaj1981.03615995004. http://dx.doi.org/10.2136/sssaj1981.03615995004.

Mk C, Davey BG (1988) Observation on some routine methods for soil pH determination. Soil Sci. 145:29–36.

Murphy JA, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Analytica chimica acta. 27:31–36.

Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture.

Pal P, Khatun N, Banerjee SK (2017) Potential screening of *Azotobacter* from soil. Int J Adv Res. 5(3):24–31.

Palad MS, Aminah (2021) The effect of biological fertilizer application on interest amount and relative water content in rehabilitation effort of old cacao. IOP Conf Ser Earth Environ Sci. 911(1):012052. doi:10.1088/1755-1315/911/1/012052. http://dx.doi.org/10.1088/1755-1315/911/1/012052.

Pavitra G, Naik B, Meghana SP, Joshi R, Nandish MS (2022) Compatibility of *Trichoderma* species with Plant Growth Promoting Rhizobacteria (PGPR). Biological Forum - An International Journal. 14(3):724–727.

Petros Kubheka B, Weldegabir Ziena L (2022) *Trichoderma*: A biofertilizer and a bio-fungicide for sustainable crop production. In: *Trichoderma* - Technology and Uses. IntechOpen.

Poveda J, Eugui D (2022) Combined use of *Trichoderma* and beneficial bacteria (mainly Bacillus and Pseudomonas): Development of microbial synergistic bio-inoculants in sustainable agriculture. Biol Control. 176(105100):105100. doi:10.1016/j.biocontrol.2022.105100. http://dx.doi.org/10.1016/j.biocontrol.2022.105100.

Püschel D, Janoušková M, Voříšková A, Gryndlerová H, Vosátka M, Jansa J (2017) Arbuscular mycorrhiza stimulates biological nitrogen fixation in two Medicago spp. Through improved phosphorus acquisition. Front Plant Sci. 8. doi:10.3389/fpls.2017.00390. http://dx.doi.org/10.3389/fpls.2017.00390.

Rao S (1977) Soil microorganisms and plant growth. New Delhi: Oxford & IBH Pub. Co.

Rueda D, Valencia G, Soria N, Rueda B, Manjunatha B, Kundapur R, Selvanayagam M (2016) Effect of *Azospirillum* spp. and *Azotobacter* spp. on the growth and yield of strawberry (*Fragaria vesca*) in hydroponic system under different nitrogen levels. J Appl Pharm Sci.:048–054. doi:10.7324/japs.2016.600108. http://dx.doi.org/10.7324/japs.2016.600108.

Siddiqui IA, Shaukat SS (2003) Combination of *Pseudomonas aeruginosa* and *Pochonia chlamydosporia* for Control of Root-Infecting Fungi in Tomato: Combination of *Pseudomonas aeruginosa* and *Pochonia chlamydosporia*. J Phytopathol (1986). 151(4):215–222. doi:10.1046/j.1439-0434.2003.00708.x. http://dx.doi.org/10.1046/j.1439-0434.2003.00708.x.

Singh O, Gupta V, Kiran H (2014) R Tewari Plant growth regulation. Springer.

Subbiah BV, Asija GL (1956) A Rapid Procedure for the Estimation of Available Nitrogen in Soils. Current Science. 25:259–260.

Sumbul A, Ansari RA, Rizvi R, Mahmood I (2020) *Azotobacter*: A potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci. 27(12):3634–3640. doi:10.1016/j.sjbs.2020.08.004. http://dx.doi.org/10.1016/j.sjbs.2020.08.004.

Tuan Muhammad Syafiq TH, Nusaibah SA, Rafii MY (2021) Effectiveness of Bioinoculants *Bacillus cereus* and *Trichoderma asperellum* as Oil Palm Seedlings Growth Promoters. Pertanika J Trop Agric Sci. 44(1). doi:10.47836/pjtas.44.1.09. http://dx.doi.org/10.47836/pjtas.44.1.09.

Velmourougane K, Prasanna R, Chawla G, Nain L, Kumar A, Saxena AK (2019) *Trichoderma-Azotobacter*biofilm inoculation improves soil nutrient availability and plant growth in wheat and cotton. J Basic Microbiol. 59(6):632–644. doi:10.1002/jobm.201900009. http://dx.doi.org/10.1002/jobm.201900009.

Vincent JM (1947) Distortion of fungal hyphæ in the presence of certain inhibitors. Nature. 159(4051):850–850. doi:10.1038/159850b0. http://dx.doi.org/10.1038/159850b0.

Vithwel D, Kanaujia SP (2014) Integrated nutrient management on productivity of carrot and fertility of soil. SAARC J Agric. 11(2):173–181. doi:10.3329/sja.v11i2.18412. http://dx.doi.org/10.3329/sja.v11i2.18412.

Wani S, Chand S, Ali T (2013) Potential Use of *Azotobacter chroococcum* in Crop Production: An Overview. Curr Agric Res J. 1(1):35–38. doi:10.12944/carj.1.1.04. http://dx.doi.org/10.12944/carj.1.1.04.

Zainuddin N, Keni MF, Ibrahim SAS, Masri MMM (2022) Effect of integrated biofertilizers with chemical fertilizers on the oil palm growth and soil microbial diversity. Biocatal Agric Biotechnol. 39(102237):102237. doi:10.1016/j.bcab.2021.102237. http://dx.doi.org/10.1016/j.bcab.2021.102237.

AUTHORS

First Author – T. T. Gunasekara, https://orcid.org/0009-0004-2479-9088 / taniyagunasekaratg11@gmail.com.
Second Author – K. K. K. Nawarathna , https://orcid.org/0000-0002-8939-4397 / kkknawaratna@yahoo.com
Third Author – A. G. B. Aruggoda , https://orcid.org/0000-0003-0222-7084 / agbar@ou.ac.lk
Fourth author – S. Kohombange, https://orcid.org/0009-0000-7289-7871 / shyamaleekohombange@gmail.com