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Climate Change Scenarios on Bioprotction Potential of Arbuscular Mycorrhizal Fungi (AMF) in Relation to Root Knot Nematode (RKN) on Tomato

E.B. Utobo, M. Uma and R. Nidhi

Department of Environmental Science, G.B. Pant Uni. of Agric. and Tech.,
Pantnagar, US Negar, Uttarankhand, 263145, India

Abstract: A study was conducted on the effect of AMF (*Glomus intraradices*), either alone or in combination with plant growth promoting rhizobacterium (PGPR; *Pseudomonas fluorescens*) on plant growth, disease status and relative disease control of RKN (*Meloidogyne incognita*) of tomato plant under climate change scenarios in Tarai region of Uttarakhand, during summer and autumn seasons of 2014. RKN were added to AMF inoculated plants with or without PGPR and were exposed to ambient conditions and to combined elevated atmospheric CO_2 concentration (+100 ppm) and temperature (+2°C) hereafter referred to "*future climate*". The main findings were: (i) shoot dry weight was significantly better in the treatment with RKN added with AMF either alone or in combination with PGPR (AMF + PGPR +RKN > AMF+RKN) than in the control and single RKN treatment and was significantly higher under "*future climate*" than under ambient climate in both seasons; (ii) disease incidence and disease index were all significantly lower in AMF + PGPR treatment when compared with AMF single treatment and were positively influenced by the "*future climate*" in the first and second seasons respectively; (iii) relative disease control was significantly higher in AMF + PGPR + RKN treatments than in AMF + RKN treatments and was significantly better under "*future climate*" when compared with ambient climate in both seasons. The results indicate that the combined application of AMF + PGPR is better in the management of RKN than AMF single treatment and the effect will be more under "*future climate*" in both seasons.

Key words: Climate change · G. intraradices · P. fluorescens · M. incognita · Tomato

INTRODUCTION

Climate change is occurring at an unprecedented rate globally, with a 2-3°C increase in mean annual temperatures, shifts in precipitation location and frequency and a 200% increase in atmospheric CO_2 predicted in some regions by the end of the 21st century [1]. Elevated CO_2 concentration and temperature, however, could seriously threaten the productivity level globally, which require mitigation strategies to cope up with the changing climate [2]. For this reason, future food security will have to be better adapted to a range of biotic and abiotic stresses to deal with the direct and indirect consequences of progressively changing climate [2]. Changes in atmospheric conditions, nutrient deposition patterns and global and regional climate affect ecosystem processes [2] and mycorrhizal functioning [3]. Yet, the potential role of mycorrhizal symbioses in mediating ecosystem responses to climate change remains underexplored [4].

Arbuscular mycorrhizal fungi (AMF) is considered an important link between below- and aboveground ecosystem processes [5] and several plant species cannot even grow or survive without their fungal symbiont [6]. AMF can receive up to 30% of a plant's photosynthates [7] and in exchange provide plants with up to 80% of their required N or P [8]. In addition, they can enhance plant drought tolerance [9] and protect plants from pathogens [10].

Corresponding Author: E.B. Utobo, Department of Environmental Science, G.B. Pant Uni. of Agric. and Tech., Pantnagar, US Negar, Uttarankhand, 263145, India.

Bharadwaj *et al.* [11] and Liu *et al*. [12] observed that the functionality of AMF is usually enhanced when applied together with other beneficial rhizosphere microbiota like plant growth promoting rhizobacteria (PGPR) than alone. The synergistic interaction between AMF and PGPR has been reported in several studies for their plant growth promoting activities [13, 14] as well as in the management of plant diseases such as root knot nematode [12, 13]. Root knot nematodes (*Meloidogyne* spp.) are widespread in distribution and are important soil borne pathogens in tomato cultivation, as well as in many economically important crops [12, 15]. Significant reduction in tomato fruit yield (34%) globally had been caused by nematodes under current production practices [16].

The direct effects of elevated CO_2 and temperature on plant development, along with potential indirect and direct effects on AMF, may alter the bio-protection potential of AMF symbionts [5, 7]. Such alterations could shift the compatibility and cooperation between hosts and fungi along the mutualism-parasitism continuum [17]; producing context-dependent responses to climate change [18]. Thus, understanding the combined effect of elevated CO_2 concentration and temperature on the functioning of AM fungi symbiosis is crucial in predicting natural and managed ecosystem responses to "*future climate"* change.

Keeping the above points in view, the study was undertaken to evaluate the impact of climate change on bio-protection potential of AMF symbiont, in Tarai region of Uttarakhand" during summer (*kharif*) and autumn (*monsoon-post monsoon*) seasons of 2014. It involved exposing tomato plants inoculated with AMF either alone or in combination with PGPR and root knot nematode (RKN) to ambient climatic conditions and to combined elevated CO_2 (+100 ppm) and temperature (+2°C) hereafter referred to as "*future climate*", using modified polyhouse chamber technology. Specifically, the following hypotheses were tested: (i) plant biomass production of mycorrhizal treated plants either alone or in combination with PGPR will be higher when compared with non-AMF control under the influence of RKN regardless of climates and seasons and the effect will be more under the influence of "*future climate*" in relation to ambient climate, in the first and second seasons respectively. (ii) the stimulatory effect of mycorrhizal treatments on plant biomass under the influence of RKN will promote the bio-protection potential of AMF irrespective of climates and seasons and it will be enhanced under "*future climate*" when compared with ambient climate in both seasons.

MATERIALS AND METHODS

Experimental Site and Soil Characteristics: The climate-controlled chamber experiments were conducted at Norman E. Borlaug Crop Research Centre, GB Pant University of Agriculture and Technology, Pantnagar, India (lat. 29.2°N, 79°E long.) during summer and autumn seasons of 2014. The climate in Pantnagar is characterised by sub-humid to sub-tropical with hot, dry summers and cool winters, with average annual air temperature (T_{air}) varying around 23°C. Annual rainfall averages 1433.4 mm unequally distributed throughout the year. The plant growth medium was a sandy loam with pH 7.7, 1.41% organic matter, 0.8 g kg^{-1} of total phosphorus and 67, 41 and 35 mg kg^{-1} available nitrogen, phosphorus and potassium, respectively.

Mass Production of AMF, PGPR and RKN: *Glomus intraradices* (AMF) spores were isolated from the trap culture by wet-sieving and decanting and were mass propagated through monosporal culture using maize (*Zea mays* L.) as a host in sterilized soil: sand (1:1) mixture, according to the modified method of Talukdar [19]. It was further produced in bulk using maize as a test plant by repeated sowing and harvesting of the plants after every 60 days for three cycles. Moisture was maintained with deionized water as and when required. The inoculum concentration was assessed by most probable number (MPN) counts [20] and the inoculum was found to contain ~50 infectious propagules (IP) /g of soil: sand (1:1) mixture. The log phase culture of *Pseudomonas fluorescens* P16 was tested for purity before multiplying on King's B (KB) broth as described by de Freitas [21] and kept at 4°C until needed. Eggs of pure culture inoculum of root-knot nematode (*Meloidogyne incognita)* maintained on tomato plants were extracted, hatched and mass propagated using tomato seedlings (TO-1458) planted in 3 kg pot containing sterilized soil-sand mixture according to modified protocol developed by Hussey and Barker [22]. The organisms were originally supplied by Dr. A.K. Sharma Rhizosphere Laboratory, Department of Biological Sciences, GBPUAT, Pantnagar, India.

Inoculation and Planting: The tomato seeds (var. T0-1458) were inoculated with AMF either alone or in combination with PGPR at sowing. Seedlings were raised using seeds bio-primed with *P. fluorescens* P16 according to Yadav *et al*. [23] protocol and control received only carboxy methyl cellulose (CMC) suspension. Sterilised soil: sand (1:1) mixture was added in plastic tray of dimension 52.5 x 27.5 x 3.5 cm (L x W x D), with 98 furrows. For AMF treatments, one gram of the fungal inoculum containing about 50 infective propagules was applied in each furrow and mixed with sterilized soil: sand (1:1) mixture. Control treatment received 1g autoclaved inoculum (free of the fungi), mixed with sterilized soil: sand (1:1) mixture. One germinated seed was then planted in each furrow and the seedlings were fertilized once a week by applying 4 mL of modified Hoagland's nutrient solution (minus P; [24]) to each furrow to replenish soil nutrients. Moisture was maintained as and when needed. At about 2 weeks after planting (WAP), the seedlings were transplanted individually with the entire furrow content into 220 g plastic cups filled with sterilised soil: sand (1:1) mixture.

For RKN treatments, the number of second stage juveniles (J_2) of root-knot nematode (*Meloidogyne incognita*) per ml of the suspension was determined (microscopic counting) before inoculations. An average of five microscopic counts was taken for determining the desired volume for inoculation. At about 1 weeks after transplanting to 220 g plastic cups (i.e. 3WAP), the seedlings were inoculated with juveniles ($J_{2)}$ of root-knot nematode. Before inoculating the plants, the nematode inoculum level was adjusted with water, by adding equal volume of nematode suspension (10ml) in each nematode treatment to obtain the desired inoculum level of 1, 000 J_2 per plant [25]. Just before inoculations, the feeder roots of the seedlings were exposed by carefully removing the top layer of the soils, the nematode suspension was thus poured uniformly all over the exposed roots and covered immediately with top soil as well as some additional sterilized soil. This was followed by light watering of the soil in the cups. A similar treatment was given to the uninoculated check plants in which only water was used instead of the nematode suspension. The seedlings were then maintained in these cups for 1 week and fertilized once with a modified Hoagland's solution. Plants were grown in a greenhouse chamber under the following conditions: 18 hours of light (600 µmol m^{-2} s^{-1}) and 27°C ± 2°C temperature and relative humidity of 60%. At 4 WAP, the seedlings were transplanted into pots of 3kg capacity filled with the unsterilized field soil. NPK (15:10:10) fertilizer was applied one week before the seedlings were transplanted.

Experimental Design and Treatments: The experimental platform consisted of four climate-controlled chambers, facing south and placed in an open area in the field where mutual shading or shading by other objects was avoided. Each chamber had an interior dimension of 1.5m x 1.5m x 2.0m, covered with transparent polyethylene films (0.2 mm thick) that was UV-transparent and allowed up to 86% light transmission. The top of each chamber was covered with green net (4mm thick) which was also UV transparent that allowed 50% light transmission, only at the peak of summer period starting from 23rd May, when maximum temperatures was exceeding 38°C. Two chambers were exposed to the ambient air temperature (T_{air}) and ambient CO_2 concentration through multiple openings (15 cm diameter). The other two chambers which have a single opening (15 cm diameter) at the top were continuously exposed to a climate within the range of +2°C air temperatures and +100 ppm CO_2 concentration above fluctuating ambient T_{air} and CO_2, hereafter referred to as "*future climate*". The CO_2 concentration in the "*future climate*" chambers was continuously maintained through injection of CO_2 from CO_2 cylinder using a 500ml syringe at an interval of 3 hours per day (9am, 12noon, 3pm and 6pm). The temperature in the "*future climate*" chambers was maintained by daytime warming above ambient and occasional night-time cooling below ambient. The outside and chamber CO_2 concentrations were collected before and after CO_2 injection in the "*future climate"* chambers with the help of 20 ml syringe. The collected CO_2 was continuously analyzed using CO_2 analyzer and average daily readings were recorded. The daily minimum and maximum outside and chamber temperatures were continuously measured with the help of thermo hygrometer and average daily readings were recorded.

The experiment had four treatments: control (CT), *M. incognita* (Mi) alone, G. *intraradices* plus *M. incognita* (Gi + Mi), *G. intraradices* plus *P. fluorescens* plus *M. incognita* (Gi + Pf + Mi). Each treatment consists of three replications and pots distribution was completely randomized in each chamber. The entire experiment was repeated in the second season.

Estimation of Plant Biomass: Shoot biomass was determined at harvest by separating the shoot from the root at the stem base, followed by washing and air drying under shade before oven-drying at 65°C for 48 hours, after which oven-dried weight was determined.

Assessment of Effectiveness of Inoculants Against Root-Knot Nematode Damage: A subsample of fine roots (5 g fresh mass per plant) was cut from the harvested plant and used for assessment of nematode galling according to a 1–5 root galling index as described by Coyne and Ross [25]: 1= no galling damage; 2 = slight galling damage; 3 = mild galling damage; 4 = heavy galling and 5 = severe galling damage. The roots were cut into 10 mm length and thirty randomly selected pieces of each sample were placed on a Petri dish marked with 0.5 cm gridlines on the bottom and observed for galls under a bright-field microscope at x50 or x125. Each intersection of root and gridline is checked for the presence or absence of galls and scored as infected or not infected by nematode. The percentage disease incidence, disease index and relative control were calculated according to method as described by Fang [26] as given blow:

$$\text{Disease incidence (\%)} = \frac{\text{Number of root segment galled per plant}}{\text{Total number of root segment per plant}} \times \frac{100}{1}$$

$$\text{Disease indexes (\%)} = \frac{\Sigma(\text{No. of root segment galled in each grade} \times \text{the represe ntative value of each grde})}{(\text{Total no. of root segment per plant} \times \text{the representative value of the highest grade})} \times \frac{100}{1}$$

$$\text{Relative control (\%)} = [1 - (\frac{\text{Disease ondex of treatment}}{\text{Disease index with Mi alone}})] \times \frac{100}{1}$$

Statistical Analysis: Data were subjected to analysis of variance (ANOVA) to determine any significant effects of the different treatments. The treatment means were compared using the least significant difference (LSD) test at a significance level of 0.05. Normality of distributions and homogeneity of variances were assessed before conducting any statistical analysis. Statistical analyses were performed using GenStat Discovery Edition software version 3.0 for Windows [27].

RESULTS AND DISCUSSION

Environmental Conditions: During the experiment, the average air temperature (T_{air}) of the ambient climate chamber (*Amb*) was 0.26°C and 0.22°C higher than average outside reference T_{air} (Standard Deviation; SD: 2.46 and 2.32), while the average T_{air} of the "*future climate*" chamber (*Fut*) was 2.21°C and 2.19°C higher than the average outside reference T_{air} (SD: 3.19 and 3.01) and the average difference between T_{air} in "*future"* and ambient climate was 1.95°C and 1.97°C (SD: 0.73 and 0.69) in the first and second seasons of the experimental year respectively. The air CO_2 concentration in the ambient climate chamber was an average of 390 and 391 ppm (SD 30.98 and 26.58), while in the "*future climate*" chamber, it was maintained within + 93 ppm and + 96 ppm of the target (490 ppm; SD 36.04 and 31.29) when compared with ambient chambers in the first and second seasons of the experimental year respectively.

Shoot Dry Weight: The analysis of variance (ANOVA) for the shoot dry mass revealed significant main effects of mycorrhizal treatments either alone or in combination with PGPR on root knot nematode and climate change scenarios in both seasons (Table 1), except for climate change scenario in season one. The shoot dry weight was significantly lower in the treatment with *M. incognita* alone than in the untreated control plant regardless of climates in both seasons, but were greater in the treatment with *M. incognita* added with *G. intraradices,* which was further enhanced with the addition of *P. fluorescens* than in the un-inoculated control plant (Fig. 1). The results suggest that AMF could partially offset the damage caused by nematodes in this tomato cultivar, as previously reported in other tomato cultivars [28]. Changes in plant biomass are in support of the detrimental effect of the nematode as oppose to the beneficial effect of the mycorrhisation on the plant. The dual inoculation of tomato plants with AMF and PGPR which resulted in more biomass production may be due to positive synergistic interactions between AMF and PGPR, as was reported by the majority of other studies [12, 13, 29]. For example, Liu *et al*. [12] observed that AMF and PGPR could interact positively to improve growth and plant nutrition and protect plants against pathogens and they are usually more effective when applied together than alone. However, the results of Medina *et al*. [30] and Vestberg *et al*. [31], found no stimulatory effect when combining these microorganisms.

The shoot dry weight was also positively affected by "*future climate*" in relation to ambient climate in the second season only of the experimental year (Fig. 1). The positive influence of "*future climate*" observed suggests an extra C flow towards the AMF, as plants allocate up to 30% of their net primary production to their fungal associates [32, 33] and in exchange AMF provide plants with up to 80% of their N or P [8].

Table 1: Statistical significance of the ANOVA for shoot dry mass, percentage disease incidence, index and relative control of tomato plant inoculated with RKN as influenced by AMF either alone or in combination with PGPR 65 days after field transplanting, under ambient climate and future [combined elevated CO_2 (+100 ppm) and warming (+2^{0}C)] climate change scenarios

	Microbial treatments (Mt)		Climate change (Cc)		Interaction (Mt x Cc)	
Parameters	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Shoot dry mass	0.000***	0.000***	0.069^{NS}	0.042*	0.973^{NS}	0.959^{NS}
Disease incidence (%)	0.000***	0.000***	0.000***	0.000***	0.015*	0.007**
Disease index (%)	0.000***	0.000***	0.001***	0.008**	0.161^{NS}	0.389^{NS}
Relative control (%)	0.000***	0.000***	0.009**	0.000***	0.071^{NS}	0.063^{NS}

Mt: Microbial treatments; Cc: Climate change scenarios; NS: No significant difference (P > 0.05); *, **, *** significant difference at P < 0.05, P < 0.01 or P < 0.001; Values are mean of three replicated (n = 3)

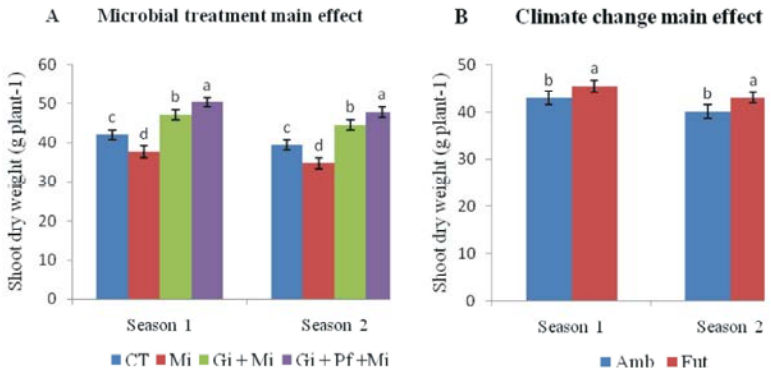

Fig. 1: Main effects of microbial treatment (A) and climate change scenarios (B) on shoot dry weight of tomato 65 days after field transplanting. CT: Untreated control; Gi: G. intraradices; Pf: Pseudomonas fluorescens; Mi: M. incognita. Means (n = 3) that do not share a letter are significantly different at P < 0.05

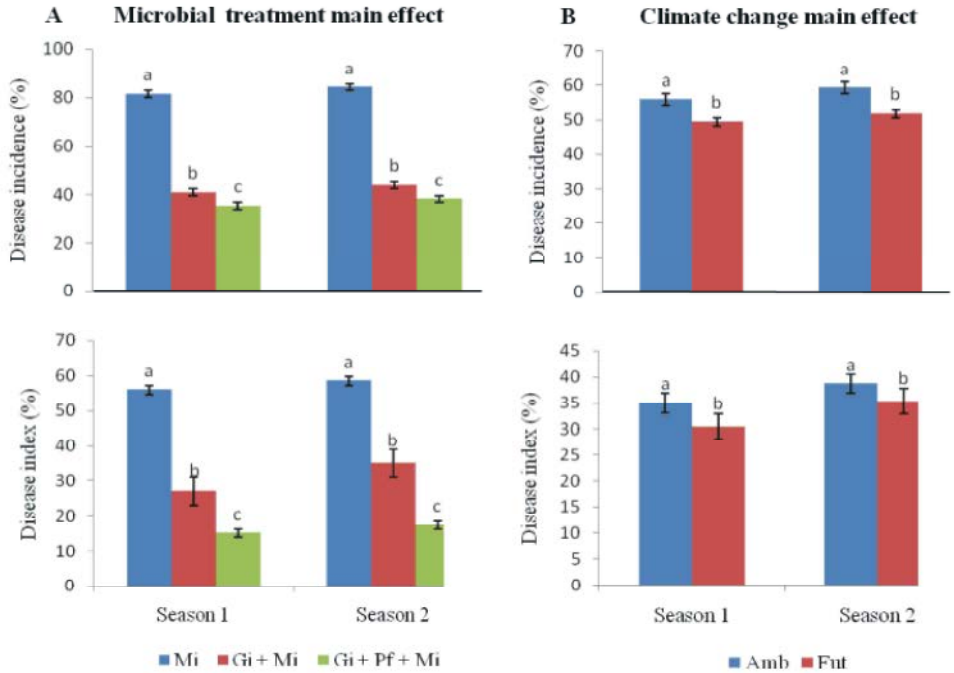

Fig. 2: Main effects of microbial treatment (A) and climate change scenarios (B) on the disease incidence (%) and the disease index (%) of tomato 65 days after field transplanting. CT: Untreated control; Gi: G. intraradices; Pf: Pseudomonas fluorescens; Mi: M. incognita. Means (n = 3) that do not share a letter are significantly different at P < 0.05

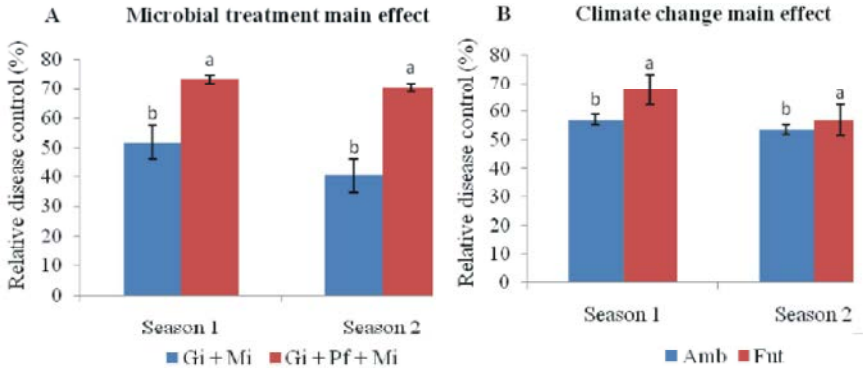

Fig. 3: Main effects of microbial treatment (A) and climate change scenarios (B) on the relative disease control (%) of tomato 65 days after field transplanting. CT: Untreated control; Gi: G. intraradices; Pf: Pseudomonas fluorescens; Mi: M. incognita. Means (n = 3) that do not share a letter are significantly different at P < 0.05

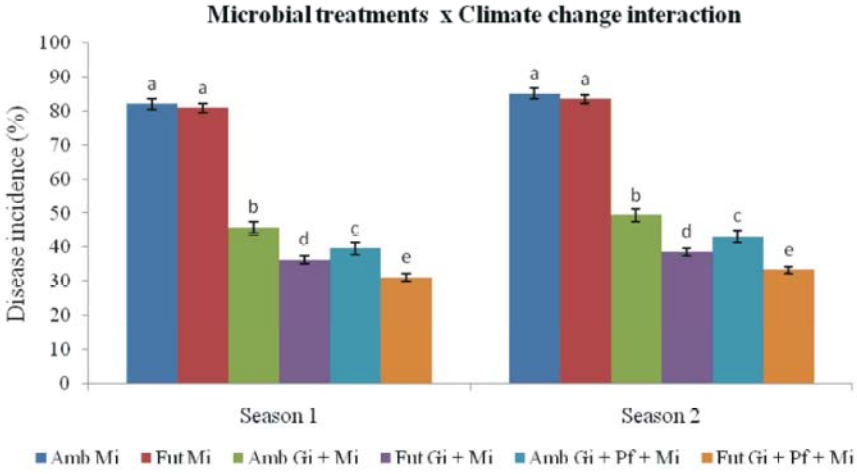

Fig. 4: Interaction effects between microbial treatments and climate change scenarios on the disease incidence (%) of tomato 65 days after field transplanting. CT: Untreated control; Gi: G. intraradices; Pf: Pseudomonas fluorescens; Mi: M. incognita. Means (n = 3) that do not share a letter are significantly different at P < 0.05

Disease Status and Relative Disease Control: Mycorrhizal treatments either alone or in combination with PGPR and climate change scenarios significantly affected the disease status and relative disease control of tomato plant inoculated with root knot nematode in the first and second seasons, respectively. In addition, there were significant interactions between microbial treatments and climate change scenarios for disease incidence in both seasons (Table 1). The plants inoculated with *G. intraradices* + *P. fluorescens* exhibited a significant reduction in term of disease incidence and disease index (Fig. 2) which led to enhancement of relative control of nematode damage (Fig. 3) compared to plants treated with *G. intraradices* alone and were all significantly better under "*future climate*" when compared with ambient climate in both seasons (Fig. 2 and Fig. 3).

The reduction of disease incidence and disease index and enhancement of relative disease control regardless of climates and seasons as a result of single mycorrhizal inoculation observed in this study, has been previously reported in other tomato cultivars [28, 34]. Furthermore Radwan *et al.* [35] observed that the disease status and relative disease control in tomato plants caused by *M. incognita* were also palliated by dual inoculation of *G. intraradices* with *P. fluorescens*, as recorded in this present study. The positive affect of "*future climate*" observed may be as a result of enhanced biomass production due to elevated CO_2 [36] and warming [37]. Staddon and Fitter [36] reported that the most significant effects of atmospheric CO_2 enrichment on AMF were expected to be indirect, through their impacts on plants. Pendall *et al.* [38] stated that where plant productivity increased with soil temperature, mycorrhizal and microbial

activity were also predicted to increase to help and meet increasing nutrient and water demands. Suzanne and Mary [17] reported that the interrelated effects of climate change factors on plants, mycorrhizae and agroecosystems, were complicated and often unpredictable. Büschera *et al*. [39] observed that in nature, CO_2 and temperature increase simultaneously, which may lead to numerous interactions.

CONCLUSION

Based on our findings, the first and second hypotheses were supported. We conclude that the combination of compatible AMF and PGPR could interact positively regardless of other factors to suppress root knot nematode on tomato plant than with AMF single treatment and their beneficial effect will be more under "*future climate*" in both seasons of *Tarai* region of Uttarakhand, India..

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