Original Research Article

Effect of arbuscular mycorrhizal fungi and bio-inoculants on germination and seedling growth of *Carica papaya* L. var. Gujarat Junagadh Papaya-1

ABSTRACT

Background: *Glomus intraradices* fungi and three bio-inoculants (*Pseudomonas fluorescens, Trichoderma viride,* and *Trichoderma harzianum*) are known to have certain beneficial effects on germination and seedling growth of several tropical fruit crops. A pot study was conducted to evaluate the effects of arbuscular mycorrhizal (AM) fungi and bio inoculants on seed germination and seedling growth of a papaya cultivar Gujarat Junagadh Papaya-1 at regional horticultural research station (RHRS), ASPEE College of Horticulture and Forestry (ACHF), Navsari Agricultural University, Navsari, Gujarat, India.

Method: The seeds of papaya were sown in the Monsoon season (June-Aug 2019) in polybags. The seeds of papaya were coated by AM fungi (*G. intraradices*), three bio inoculants, and their combinations. There were 9 treatments including control treatment.

Result: An application of *T. harzianum* was found to be the most effective on seed germination and seedling growth by enhancing germination percentage (89 %) along with early emergence (6 days) and consolidating all the growth parameters. *T. harzanium* was also effective on underground plant parts such as taproot length and diameter. The combination of all four bio-inoculants also gave significant results. Results from this study could be helpful for the West Indian fruit growers by enhancing the opportunities to use bioagents for sustaining their farming systems.

Comment [iU1]: Elaborate your best results of experiment properly and also suggest which treatment was the best one amongst all

Key words: Papaya, *Glomus intraradices*, *Pseudomonas fluorescens*, *Trichoderma viride*, and *Trichoderma harzianum*, GJP-1

INTRODUCTION

Seed is one of the most important and prime elements in increasing agricultural production in farming system, thus the higher quality of seed should be sown. The seed quality can be achieved by various methods but seed treatment is the best method for getting the higher productivity and plant population. From the different seed treatments, pre-sowing management with mycorrhiza and bio inoculants have proved very effective in controlling the pathogens and increasing the seedling vigour. However, use of effectual microorganisms as a pre-sowing seed treating agent is apprized to be eco-friendly and favorable to both seed and environment. In general, 80-90 % germination has been recorded in many crops by using of these inoculants (Bhavya et al., 2017). The application of inoculants to the seeds is not only increased germination but also effect on growth of seedlings (Rao, 2007).Sometime this bio inoculants use as bio control agents also for nursery disease (Rangel et al.,2016). The demand for quality seeds of well-established varieties has increased with the commercialization of papaya cultivation. But due to sarcotesta (a gelatinous material covering the seed coat), the germination of papaya is slow, and it needs to be fastened to increase the germination rate. As a remedial measure, the removal of sarcotesta is essential. These hindrance factors are present in freshly extracted seeds which reduce the germination rate while dried seeds tend to germinate well

Comment [iU2]: Include a short paragraph of ***Carica papaya***

(Tokuhisa et al., 2007). According to a previous study, seed treatment with *P. fluorescens* and *T. harzianum* suppress the infestation of nematode that could lead to improved germination and proper growth of seedlings (Rao, 2007). *Trichoderma* species have been acknowledged as biocontrol agents and plant growth stimulators, opted for seed treatments to reduce the pathological infestations, and enhance seedling vigor (Lingyun et al., 2017) *Trichoderma spp.* (free-living fungi) are usually found in soil and root ecosystems.

The slow germination of seeds is due to the presence of some inhibitors like phenolic compounds and pathogenic attacks. Seed treatment is required to promote seed germination, seedling growth, and reduce the germination time with suitable growing media. Based on the information, it was said that to introduce the endomycorrhiza and bio inoculants as a seed coating which makes the symbiotic relation between the seedling and fungus for better seedling growth. Thus, the present investigation was contemplated to find out the advisable endomycorrhiza for effective seed treatment and the effect of these inoculants on the survival rate in the papaya seed.

MATERIALS AND METHODS

An experiment was carried out in June 2019 at regional horticultural research station (RHRS), ASPEE College of Horticulture and Forestry (ACHF), Navsari Agricultural University, Navsari, Gujarat, India (20º 57 ′ N latitude and 72º 54 ′ E longitude with an altitude of about 11.89 m above mean sea level (MSL)) in well maintain polyhouse.

According to the agro-climatic situation, Navsari falls in South Gujarat Heavy Rainfall Zone -I and Agro-Ecological Situation-III (AES-III). The climate of this zone is tropical, characterized by humid and warm monsoon with heavy rainfall, moderately cold winter, and fairly hot and humid summer. The monsoon set from the second week of June and withdrawal by September end. The intensity of rainfall is high during July and August months. The winter season starts in November and ends in the middle of February. December and January are the coldest months, whereas April and May are the hottest. Mean weekly temperatures and rainfall during the experimental period (19th June 2019 to 2nd August 2019) are shown in (Figure 1).

The experiment was carried out in a complete randomized design (CRD) with three replications. The treatments were randomly allotted to different plots. The polythene bags were kept in polyhouse and arranged into 9 sets representing treatments with 50 bags in each set. The media mixture of laterite soil + farmyard manure + vermicompost + coco peat (1:1:1:1) was filled in polythene bags (6″ × 2 ″ size) 50-micron having longevity holes for drainage of excess water. In each polybag, one seed was sown at 1 cm depth on 19th June 2019 and covered by a shallow layer of media. Optimal moisture in the media was maintained by a sprinkling of water twice a day until the emergence of germination started. Once germination was started, the sprinkling of water was done once a day. After 50 % germination, watering was done on alternate days to maintain optimum moisture level.

There were 9 treatments viz. *G. intraradices* 10 g (T1), *P. fluorescens* 10 ml (T2), *T. viride* 10 g (T3), *T. harzianum* 10 g (T4), T1 + T2 [*G. intraradices* (10 g) + *P. fluorescens* (10 ml)] (T5), T1 + T3 [*G. intraradices* (10 g) + *T. viride* (10 g)] (T6), T1 + T4 [*G. intraradices* (10 g) + *T. harzianum* (10 g)] (T7), T1 + T2 + T3 + T4 [*G. intraradices* (10 g) + *P. fluorescens* (10 ml) + *T. viride* (10 g) + *T. harzianum* (10 g)] (T8) and control (T9). The application of AMF and bio-inoculants has been done as per treatments. For this, slurry in the ratio of 1:2 (water: fungus powder) was prepared and seeds were soaked in slurry until uniformly covered. These seeds were dried in shade for 5-7 minutes and used for sowing in the polythene bags according to the allotted treatment. Light irrigation was given to each polybag after seed sowing.

The seeds of GJP- 1 were procured from Junagadh Agriculture University, Junagadh, and Gujarat, India. Gujarat Junagadh Papaya – 1 (GJP-1) developed through the mass selection which is hermaphrodite and resistant to ringspot virus (Anonymous, 2017).

Germination parameters such as days of emergence and germination percentage were observed. In each treatment, the date of first plumule emergence and the number of days from the date

of sowing to the date of plumule emergence were recorded and expressed as the number of days taken for the emergence of seed. The germination percent was calculated by GP = (Total germinated seed)/ (Total number of seed) (Ashraf and Foolad, 2005).

Above ground parameters such as seedling height, stem diameter, and the number of leaves of previously tagged seedlings were measured in centimeter by using meter scale from the base to the tip of seedling, millimeter by using Vernier caliper respectively, while the number of leaves was calculated manually at 30 and 45 days after sowing (DAS) and mean height of seedling was calculated. Leaf area was measured using leaf area meter and Chlorophyll content of leaf was measured as per method given by (Sadasivam and Manickam, 1997).

Below ground parameters such as the number of secondary roots, taproot length, and taproot diameter were measured by uprooting and removing adhered soil particles. Length of taproot was measured with centimeter-scale, taproot diameter was measured with Vernier caliper and secondary roots were calculated manually, after 45 DAS and the average value was calculated for each treatment.

For the fresh weight of shoot and root, seedlings were uprooted carefully without damaging roots. The soil ball of the seedlings was dipped for 10 minutes in a bucket containing water to remove adhered soil particles. Roots were further dipped in clean water in order to enhance the accuracy of the fresh weight of the root. The shoots and roots of these seedlings were then separated with the secateurs from the collar region and weighed immediately on an electronic weighing balance and the mean fresh weight of shoot and root were recorded in each treatment.

For the dry weight of shoots and roots, all the seedlings were chopped and kept in a punched brown paper bag. All the bags were labeled for future identification and then placed in a hot air oven. The shoots and roots were dried in an oven at 60º C till the constant dry weight was attained. Then the dried shoots and roots were weighed separately on an electronic weighing balance and the average weight was calculated. The shoot: root fry weight ratio was calculated by dividing the fresh weight of shoot and root.

At the end of the experiment (45 DAS), the survival percentage was calculated of each experimental unit by calculating the total number of seedlings and survived seedlings.

Statistical analyses: The experimental data were subjected to one-way ANOVA in GraphPad Prism 8.3.1 549 (GraphPad Software, Inc., San Diego, California) and Kruskal-Wallis and Friedman test was used. Non-transformed data were utilized for analysis as data transformation did not improve the homogeneity of variance significantly. The treatment differences were tested by the F-test of significance based on the null hypothesis. The appropriate standard error of the mean (S. Em ±) was calculated in each treatment and the treatment means were compared with a 5 percent probability level.

RESULT AND DISCUSSION

Influence on germination and survival parameters: The seeds of papaya were affected by different seed treatments; however, the substantial difference in the production of growth promoters between the different treatments is likely to be responsible for inducing the differences in days taken to emergence, germination percentage, and survival percentage (Figure 2). Seed emergence was significantly ($p < 0.0001$) 6 days earlier in *T. harzianum* along with maximum germination ($p < 0.0001$) (89 %) and survival ($p = 0.0013$) (92.43 %) percentage than control. In particular, early seed emergence and germination treatment T8 and T6 were also found effective in certain growth parameters, the same as in survival percentages T8, T6 and T5 found effective.

An increase in germination attributing characters with seed treatments of *T. harzianum* might have induced *Trichoderma* strains that produce cytokinin-like molecules or gibberellic acid. The controlled production of these compounds could improve metabolic activities which lead to enhanced seed germination. This type of finding was observed in tomato (Gravel et al., 2007), maize (Harman, 2006) and rice (Doni et al., 2014). *Trichoderma* generates growth components that accelerate seed germination and also compete with other microorganisms for key exudates that stimulate the germination of propagules of plant-pathogenic fungi in soil (Howell, 2002). *Trichoderma* not only promotes seedling growth and vigor but also acts as a bio-control agent. During seed germination, the successful colonization of *Trichoderma* helps in reducing the pathogenic attack on the plant due to its plant defensive mechanisms and mycoparasitism effect (Yaqub and Shahzad, 2008:Vinale et al., 2008)**.**

Influence on shoot parameters: The different seed treatments influenced seedling height, stem diameter, fresh weight of shoot, and dry weight of shoot, at 30 and 45 DAS (Table 1). In both seedling height and stem diameter at 30 and 45 DAS, the mean was significantly ($p < 0.0001$) higher in *T. harzianum* than in control. Treatments T8 and T6 had observed similar effects as *T. harzianum*. Moreover, the fresh and dry weight of shoot was also significantly higher in *T. harzianum* (T4) at 45 DAS.

The growth of plants initiates in the meristematic regions through cell division, elongation, and differentiation. After cell division, the daughter cell enlarges to the size of the mother cell before they divide. During this cell enlargement and maturation, there has been elevated uptake of water and solute absorption (Prajapati et al., 2007). This process of cell division and elongation leads to an increase in height and stem diameter. Also, nitrogen-fixing nature and the ability to solubilize the nutrients by *Trichoderma spp*. might be responsible for increasing seedling height and stem diameter. The mechanism of phosphate solubilization by *Trichoderma* involves acidification of the medium by the production of organic acids including acetic, butyric, citric, and fumaric. The other processes include chelation, reduction, and enzymatic degradation of complex organic phosphorus compounds (acid and alkaline phosphatases) (Daangi, 2016). These findings are in accordance with the result of apple (Raman, 2012), banana (Ramesh and Ramassamy, 2015), aonla (Mandal et al., 2013), and papaya (Chandra, 2014). Root size was increasing by translocation of food acropetally leads to increases in shoot size, which translates into the increase of shoot biomass (Akladious and Abbas, 2014).

Influence on leaf parameters: Interaction of number of leaves ($p < 0.0001$), Chlorophyll content ($p = 0.0013$) and leaf area ($p < 0.0001$) was significant (Figure 3 and 4). The effect of *T. harzianum* was maximum on the number of leaves (8.20 and 13.03 at 30 and 45 DAS, respectively), Chlorophyll content (1.36 mg/g), and leaf area (136.67 cm^2) as compared to other treatments. On the other hand, none of the assessed parameters were affected by control treatment. The number of leaves and seedlings exhibited a similar pattern with T4 in T8. However, there was a significant ($p < 0.0001$) difference observed in terms of chlorophyll content in multiple comparisons between T4, T8, and T3.

A higher number of leaves leads to more food accumulation by higher chlorophyll content and escalate photosynthesis rate (Mastouri et al., 2010). *Trichoderma spp*. are known to have beneficial effects on plant growth, especially on leaf development by producing phytohormones, solubilizing insoluble nutrients in the soil (Sajeesh, P.K., 2015; Halifu et al., 2019). Trichoderma improves Mg uptake which is a key element in chlorophyll constituent and also involved in catalyzing enzymatic activity as well as in regulating photosynthesis (Doni et al., 2014).

Influence on root parameters: All the root parameters including taproot length, taproot diameter, number of secondary roots, fresh and dry weight of root, and shoot: root ratio were significantly influenced by the application of *T. harzianun* (Table 2). Taproot length and diameter were similarly influenced by T8 and T3 as by T4. T8, T3, and T1 treatments were not significant with T4 in terms of numbers of secondary roots, fresh and dry weight of root and shoot: root ratio.

These results are possible due to the ability of *Trichoderma* to produce phytochromes such as Indol Acetic Acid (IAA) and their analogous, vitamins, and enzymes. Phytochromes, vitamins, and enzymes produced by *Trichoderma* are considered responsible for stronger as well as longer root growth, increasing root diameter, and several secondary roots (Vinale et al., 2008; Vinale et al., 2012). These types of similar results were previously observed in Arabidopsis (Contreras-Cornejo et al., 2009), rice (Doni et. al., 2014), beans (Hoyos-Carvajal et al., 2009), and tomato (Samolski et al., 2012). The maximum root bio-mass found in *T. harzianum* was might be due to the modification of root architecture by the induction of auxin. This leads to the enlargement of the total absorptive surface of the roots, thereby facilitating the root growth (Samolski et al., 2012). This was earlier observed in rice (Zaidi et al., 2014) and maize (Akladious and Abbas, 2014).

CONCLUSION

The effects of different bio inoculants on germination and seedling growth of papaya were evaluated in this study. The highest germination and survival rate with early seed emergence was achieved by *T. harzianum*. Similarly, the performance of other tested growth parameters was also improved in this same treatment compared to other treatments. Therefore, this study recommends 10 g *T. harzianum* 1 kg of papaya seed in a ratio of 1: 2 (water: *T. harzianum*) for the optimum health of papaya seedlings. Considering the systemic resistant nature of *T. harzianum*, it could use as fertilizer that can enhance the resilience of organic papaya production in an open field.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

Akladious SA and Abbas SM. (2014). Application of *Trichoderma harzianum* T22 as a biofertilizer potential in maize growth. *Journal of plant nutrition*. **37**(1): 30-49.

Anonymous.2017. http://www.jau.in.

Ashraf M and Foolad MR. (2005). Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non- saline conditions.*Advance in Agronimy*. **88**: 223-271.

Bhavya, N.; Naik, N.; Masuthi, D. and Sabarad, A. (2017). Studies on effect of different storage conditions on viability of karonda seeds. *International journal of current microbiology and applied sciences*. **6**(9): 1057-1066.

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C and López-Bucio J. (2009). *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Plant Physiology.*,**149**(3): 1579-1592.

Daangi TS. 2016. *Effect of Integrated Nutrient Management on Growth, Yield and Quality of Ber (Zizyphus mauritiana Lamk.) cv. Seb, 91-92.*M.Sc. Diss., K. N. K. College of Horticulture, 2016. 112 pp.

Doni, F.; Isahak, A.; Zain, C.R.C.M.; Ariffin, S.M.; Mohamad, W.N.W. and Yusoff, W.M.W.(2014). Formulation of *Trichoderma sp.* SL2 inoculants using different carriers for soil treatment in rice seedling growth. *SpringerPlus*.,**3**: 532.

Doni F, Anizan I, Radziah CC, Salman AH, Rodzihan MH and Yusoff WMW. 2014. Enhancement of Rice Seed Germination and Vigour by *Trichoderma spp. Journal of Applied Science, Engineering and Technology.* **7**(21): 4547-4552.

Gravel V, Antoun H and Tweddell RJ. (2007). Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry.* **39**(8): 1 968-1977.

Halifu, S.; Deng, X.; Song, X. and Song, R. (2019). Effects of Two Trichoderma Strains on Plant Growth, Rhizosphere Soil Nutrients, and Fungal Community of Pinus sylvestris var. *Mongolica* Annual Seedlings. *Forests*.**10**: 758

Harman GE. (2006). Overview of mechanisms and uses of *Trichoderma spp*. *Phytopathology.* **96**(2): 190-194.

Howell C. (2002). Cotton seedling preemergence damping-off incited by *Rhizopus oryzae* and *Pythium spp*. and its biological control with *Trichoderma spp. Phytopathology.* **92**(2): 177-180.

Hoyos-Carvajal L, Orduz S and Bissett J. (2009). Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biological Control.* **51**(3): 409-416.

Lingyun W, Dongwei Y and Ming L. (2017). Effects of solid matrix priming with *Trichoderma harzianum* on seed germination, seedling emergence and photosynthetic capacity of eggplant. *African Journal of Biotechnology.* **16**(14): 699-703.

Mandal K, Rajak A, Debnath S and Hasan M. (2013). Integrated nutrient management in Aonla cv. A-7 in the red lateritic region of West Bengal. *Journal of Crop and Weed.*, **9**(1): 121-123.

Mastouri F, Björkman T and Harman GE. (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology.* **100**(11): 1213-1221.

Prajapati ND, Purohit S, Sharma A. and Kumar T.(2007). *A handbook of medicinal plants*: *A Complete Source Book.* Jodhpur, India.

Raman J. (2012). Response of Azotobacter, *Pseudomonas* and *Trichoderma* on growth of apple seedling. *International Conference on biological and life sciences IPCBEE, IACSIT Press*, Singapore. **40**: 83-90.

Ramesh Y and Ramassamy V. (2015). Influence of *Pseudomonas fluorescens* as Biofertilizer in Secondary Hardening of Tissue Cultured Banana Var. Poovan. *International Journal of Applied Sciences and Biotechnology*.**3**(1): 38-41.

Rangel, J. C. S.; Gomez, C. J.; Sanchez, G. M.; Torres, M. A. B.; Ochoa, J. M. and Cupul, W. C. (2016). Biocontrol of damping off diseases in Carica papaya seedlings under greenhouse conditions using *Trichoderma spp*. *3rd Biotech. Summit, Mexico*,127-132.

Rao M. (2007). Papaya seedlings colonized by the bio-agents *Trichoderma harzianum* and *Pseudomonas fluorescens* to control root-knot nematodes. *Nematologia Mediterranea.*,**35**(2): 199-203.

Sadasivam S and Manickam A. (1997). *Biochemical methods*. Second Edition. New Delhi.

Sajeesh, P.K. (2015). *Cu-Chi-Tri: A Triple Combination for the Management of Late Blight Disease of Potato (Solanum tuberosum L.).* Ph.D. Diss., GB Pant University of Agriculture and Technology, Pantnagar, India, 278 pp.

Samolski I, Rincon AM, Pinzón LM, Viterbo A and Monte E. (2012). The qid74 gene from Trichoderma harzianum has a role in root architecture and plant biofertilization. *Microbiology.***158**(1): 129-138.

Tokuhisa, D., Dias, D.C.F.S., Alvarenga, E.M., Hilst, P.C. and Demuner, A.J. (2007). Penolics inhibitors of germination in papaya seeds. *Brazilian Seed Magazine*. **29**: 161-168.

Vinale F, Sivasithamparam K, Ghisalberti E, Marra R, Barbetti M, Li H, Woo S and Lorito M. (2008). A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiological and Molecular Plant Pathology.* **72**(1-3): 80-86.

Vinale F, Sivasithamparam K, Ghisalberti EL, Ruocco M, Woo S and Lorito M. (2012). *Trichoderma* secondary metabolites that affect plant metabolism. *Natural product communications.***7**(11): 1545-1550.

Yaqub F and Shahzad S. (2008). Effect of seed pelleting with *Trichoderma spp*., and *Gliocladium virens* on growth and colonization of roots of sunflower and mung bean by *Sclerotium rolfsii*. *Pakistan Journal of Botany*.**40**(2): 947-953.

Zaidi NW, Dar MH, Singh S and Singh U. (2014). *Trichoderma* species as abiotic stress relievers in plants. *Biotechnology and Biology of Trichoderma*. *Elsevier* 515-525.

Table 1: Effect of seed treatments on seedling height, stem diameter, fresh and dry weight of shoots

Treatments	seedling height (cm)		Stem diameter (mm)		Fresh weight of shoot (g)	Dry weight of shoot
	30 DAS	45 DAS	30 DAS	45 DAS		
T_1: *G. intraradices* 10 g	8.70 ± 0.06	16.57 ± 0.68	3.56 ± 0.07	9.20 ± 0.35	6.82 ± 0.11	0.64 ± 0.02
T_2: *P. fluorescens* 10 ml	8.76 ± 0.03	14.77 ± 0.19	3.40 ± 0.06	8.68 ± 0.09	6.96 ± 0.28	0.67 ± 0.01
T_3: *T. viride* 10 g	8.83 ± 0.03	17.63 ± 0.32	3.80 ± 0.12	9.31 ± 0.31	8.58 ± 0.01	0.83 ± 0.02
T_4: *T. harzianum* 10 g	9.47 ± 0.12	19.80 ± 0.21	4.20 ± 0.06	9.92 ± 0.03	9.05 ± 0.18	0.86 ± 0.01
T_5: $T_1 + T_2$ [*G. intraradices* (10 g) + *P. fluorescens* (10 ml)]	8.77 ± 0.09	16.67 ± 0.38	3.73 ± 0.12	9.26 ± 0.02	8.38 ± 0.02	0.81 ± 0.01
T_6: $T_1 + T_3$ [*G. intraradices* (10 g) + *T. viride* (10 g)]	9.10 ± 0.36	18.76 ± 0.56	3.96 ± 0.03	9.64 ± 0.33	7.10 ± 0.25	0.63 ± 0.01
T_7: $T_1 + T_4$ [*G. intraradices* (10 g) + *T. harzianum* (10 g)]	8.73 ± 0.12	14.37 ± 0.47	3.16 ± 0.12	8.33 ± 0.06	6.86 ± 0.27	0.72 ± 0.01
T_8: $T_1 + T_2 + T_3 + T_4$ [*G. intraradices* (10 g) + *P. fluorescens* (10 ml) + *T. viride* (10 g) + *T. harzianum* (10 g)]	9.37 ± 0.17	18.77 ± 0.16	4.13 ± 0.07	9.79 ± 0.01	8.60 ± 0.30	0.82 ± 0.01
T_9: Control	8.60 ± 0.10	13.63 ± 0.23	3.10 ± 0.06	8.09 ± 0.04	6.05 ± 0.01	0.61 ± 0.03
SEm	0.15	0.39	0.083	0.195	0.19	0.015
CD ($P \leq 0.0001$)	0.45	1.16	0.247	0.581	0.58	0.047

G. intraradices: *Glomus intraradices*; *P. fluorescens*: *Psudomonas fluorescens*; *T. viride* : *Trichoderma viride* ; *T. harzianum* : *Trichoderma harzianum* ; ** $p \leq 0.0001$, very significant; **** $p \leq 0.0001$, Sig., Extreme Significance

Comment [iU3]: Correct table 1

Table 2: Effect of seed treatments on taproot length, taproot diameter, no. of secondary roots, fresh and dry weight of root, shoot: root ratio

Treatment	Tap root length (cm)	Tap root diameter (mm)	Number of secondary roots	Fresh weight of root (g)	Dry weight of root (g)	Shoot: root ratio
T_1: *G. intraradices* 10 g	12.35 ± 0.12	7.73 ± 0.07	12.00 ± 0.12	1.97 ± 0.06	0.29 ± 0.01	3.46 ± 0.05
T_2: *P. fluorescens* 10 ml	9.75 ± 0.06	6.67 ± 0.12	10.80 ± 0.23	2.07 ± 0.03	0.32 ± 0.01	3.37 ± 0.13
T_3: *T. viride* 10 g	13.52 ± 0.31	8.15 ± 0.10	12.13 ± 0.24	2.16 ± 0.01	0.38 ± 0.01	3.97 ± 0.01
T_4: *T. harzianum* 10 g	14.03 ± 0.45	8.50 ± 0.17	12.80 ± 0.23	2.25 ± 0.01	0.39 ± 0.01	4.01 ± 0.07
T_5: $T_1 + T_2$ [*G. intraradices* (10 g) + *P. fluorescens* (10 ml)]	11.30 ± 0.32	7.57 ± 0.17	11.90 ± 0.15	2.12 ± 0.01	0.37 ± 0.01	3.94 ± 0.03
T_6: $T_1 + T_3$ [*G. intraradices* (10 g) + *T. viride* (10 g)]	9.37 ± 0.22	6.23 ± 0.19	10.80 ± 0.53	2.09 ± 0.02	0.26 ± 0.01	3.38 ± 0.13
T_7: $T_1 + T_4$ [*G. intraradices* (10 g) + *T. harzianum* (10 g)]	10.08 ± 0.33	7.13 ± 0.12	11.07 ± 0.41	1.95 ± 0.08	0.35 ± 0.01	3.53 ± 0.24
T_8: $T_1 + T_2 + T_3 + T_4$ [*G. intraradices* (10 g) + *P. fluorescens* (10 ml) + *T. viride* (10 g) + *T. harzianum* (10 g)]	13.95 ± 0.13	8.20 ± 0.17	12.67 ± 0.13	2.17 ± 0.01	0.38 ± 0.01	3.96 ± 0.13
T_9: Control	8.70 ± 0.60	6.07 ± 0.03	10.67 ± 0.24	1.81 ± 0.10	0.25 ± 0.00	3.35 ± 0.17
SEm	0.33	0.13	0.28	0.046	0.009	0.13
CD ($P \leq 0.0001$)	0.97	0.40	0.84	0.138	0.027	0.37

G. intraradices: Glomus intraradices; P. fluorescens: Psudomonas fluorescens; T. viride : Trichoderma viride ; T. harzianum : Trichoderma harzianum ; **** $p \leq 0.0001$, Sig., Extreme Significance, ** $p = 0.0014$,

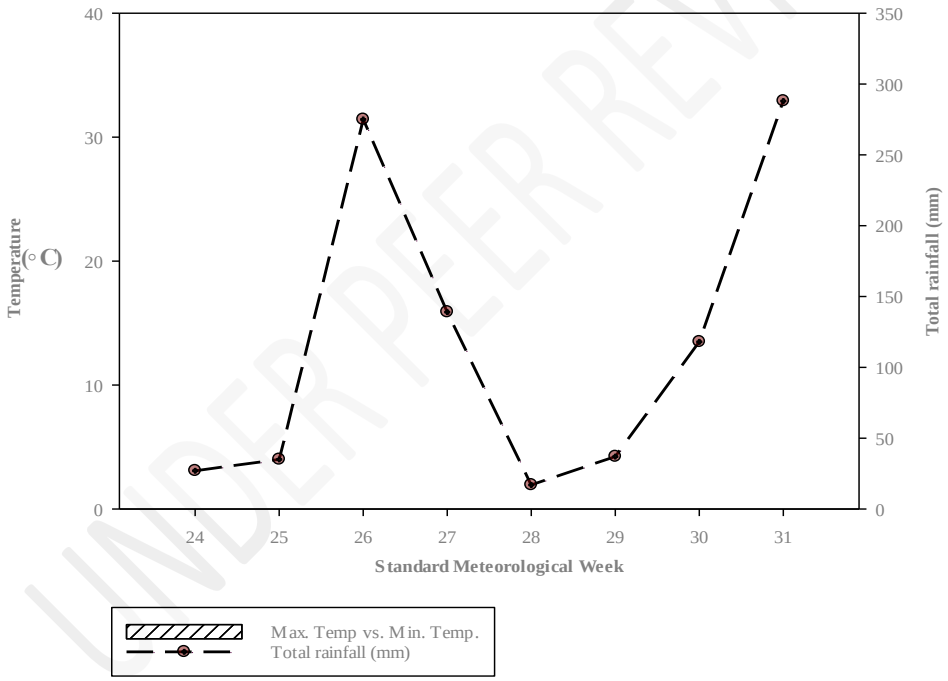

Figure 1: Mean weekly temperature and the total rainfall during the whole experiment 2019.

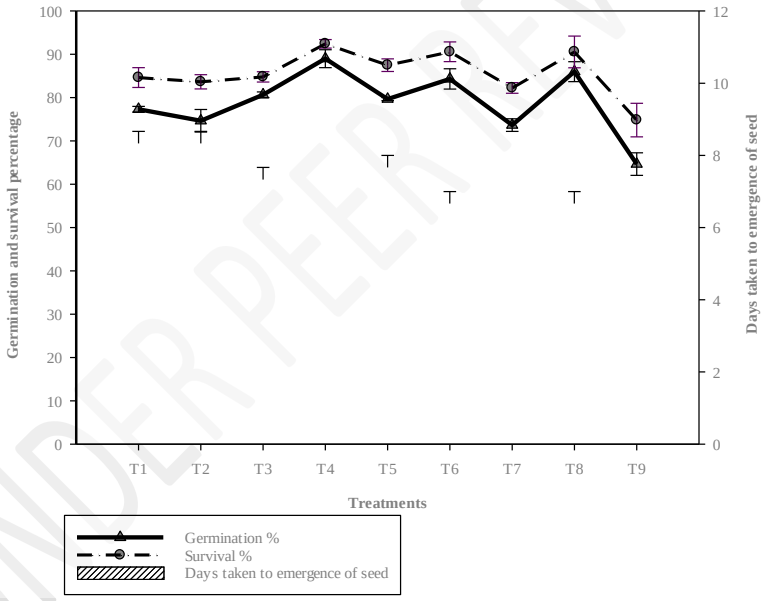

Figure 2: Effect of seed treatment on Days taken to the emergence of seed, germination percentage, and survival percentage.

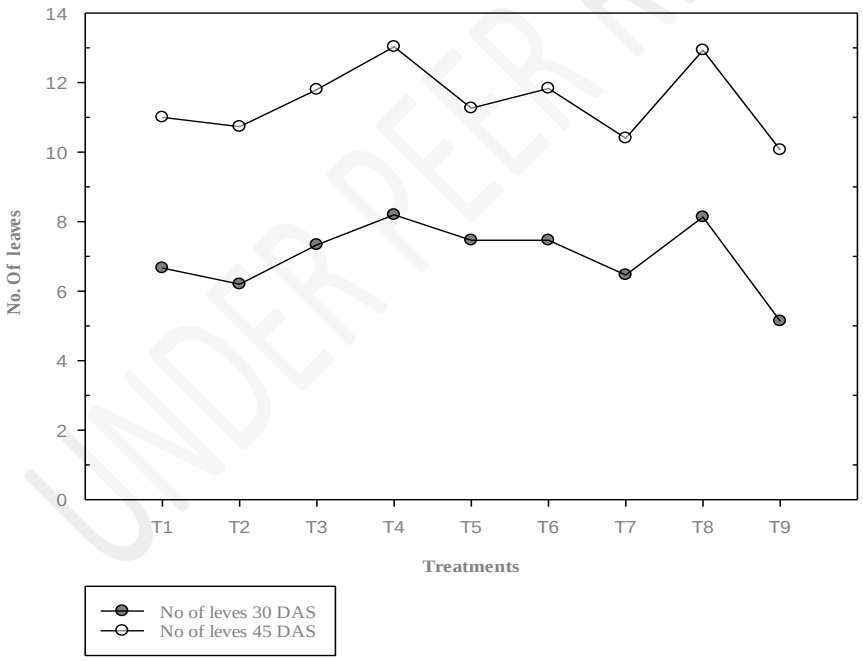

Figure 3: Effect of seed treatments on the number of leaves/seedlings at 30 and 45 DAS

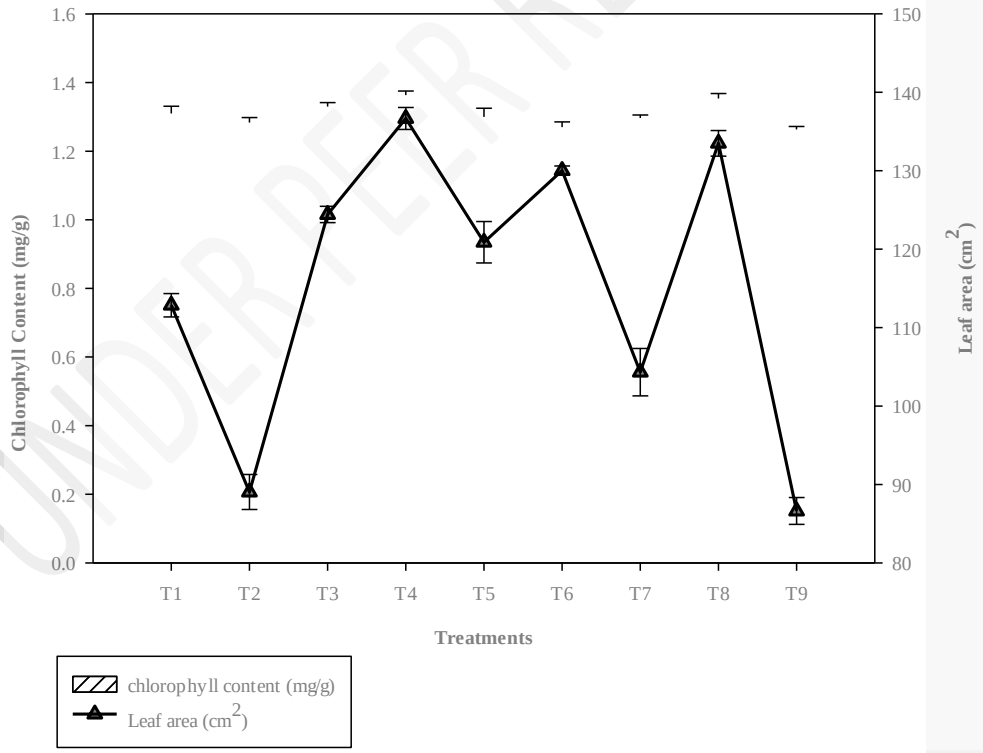

Figure 4: Effect of seed treatments on Chlorophyll content and leaf area at 45 DAS