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Evaluation of bio-fertilizers on seed germination and vegetative growth of Kachai Lemon (*Citrus jambhiri* Lush.) under low cost hydroponic condition

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Abstract
An experiment entitled "Evaluation of bio-fertilizers on seed germination and vegetative growth of Kachai Lemon (*Citrus jambhiri* Lush.) Under low cost hydroponic condition" was conducted at Department of Horticulture, College of Agriculture, CAU, Imphal, Manipur during the year 2021-22. The experiment was laid out in Completely Randomized Design (CRD) with ten treatments replicated three times. Seed treatment with different bio-fertilizers comprising of *Pseudomonas fluorescens* (5 g/kg of seeds), *Azotobacter* (5 ml/kg of seeds), *Azospirillum* (5 ml/kg of seeds), VAM (5 ml/kg of seeds), *Trichoderma viride* (5 ml/kg of seeds) and their combination with *Trichoderma viride* was done after seed extraction and washing, which were later sown on low cost hydroponic frames using blotting papers and bamboo, installed on plastic trays filled with distilled water. The observations were recorded at 4 months after sowing and it was revealed that the application of treatment T_7 - *Azotobacter* + *Trichoderma viride* recorded the minimum days taken for 50% germination (62.43 days) and complete germination (87.09 days) as well as the highest germination percentage (91.67%), germination vigour index (0.63), plant height (7.63 cm), leaf area/plant (2.10 cm^2), root length (8.97 cm), seedling vigour index-I (1522), seedling vigour index-II (17.33), fresh weight of shoot (0.240 g) and root (0.054 g), and dry weight of shoot (0.163 g) and root (0.026 g). While T_5 -*Trichoderma viride* had given lowest incidence of fungal infection (1.67%) during the germination stage.

Keywords: Kachai Lemon, bio-fertilizers, CRD, seed germination, shoot growth, root growth

Introduction
The Kachai Lemon (*Citrus jambhiri* Lush.), a species of rough lemon that is native to Kachai village in the Ukhrul District of Manipur, India, is a high yielding landrace of rough lemon with an equally high economic value (Anon., 2020; Subramanian, 2020) [2, 40]. It is locally known as Kachai Champra in Manipur. Fruit surface is smoother than other rough lemon surface, which is harsh. The weight of a single fruit varies from 60 to 100 g. Flowering starts from February up to April and the fruit matures and ripens during the month of December and January which is also the peak harvest period. The TSS content is 6.8 -10.5 brix. Juice content varies from 40 to 57 percent depending on the fruit (Datta *et al*., 2020; Anon., 2020) [12, 2]. The Kachai Lemon is distinguished from other lemon varieties grown in other parts of India by having a high ascorbic acid content of 46-51% or more, the highest so far currently available in the realm of citrus fruits while other lemon varieties only contain 20–40% of ascorbic acid. The uniqueness of Kachai Lemon which is commonly known as the "Pride of Manipur" was rewarded the prestigious geographical indication (GI-446) tag in 2014 (Anon., 2014) [1]. Kachai Lemon may be tried as potential source of rootstock for lemons, limes, sweet oranges, mandarins, and grapefruits. In addition, it is tolerant to salt and drought (Cimen and Yesiloglu, 2016) [11], and the Citrus Tristeza Virus (Savita and Nagpal, 2012) [32], Citrus Exocortis Viroid (Hardy *et al*., 2008) [17] as well as Citrus Xyloporosis Viroid (Singh *et al*., 2018) [36]. Therefore, it is propagated by seeds for rootstock purposes. Moreover, vegetative propagation techniques like budding and grafting may be standardised in order to see the response of rootstocks with readily available scions (Dilip Singh *et al*., 2022) [14].
Due to the recalcitrant nature of citrus seed, 100% germination is difficult and poor under field condition. It is reported that minimum germination percentage of rough lemon (Sharma and Dhaliwal, 2013) [34] and Kachai Lemon (Singh *et al*., 2022) [37] were observed in open field condition.

However, as reported by Singh *et al*. (2016) [28], Rangpur Lime germination began after 13 days using the hydroponic technique of seed germination, with a germination rate of 98.9%, as compared to 21.4% in the field condition, which sprouted after 30-35 days of sowing. For obtaining high quality and healthy saplings seed treatment with different bio-fertilizers can be an alternative method for successful propagation of Kachai Lemon. They are known to fix atmospheric nitrogen, solubilise the insoluble forms of phosphates, produces growth hormones, anti-microbial substances, siderophores etc. which are responsible for suppressing phytopathogens, etc., ultimately promoting plant development and therefore, enhancing plant health (Hazarika and Ansari, 2007; Asoegwu *et al*., 2020) [18, 5].

Materials and Methods

The present investigation entitled "Evaluation of bio-fertilizers on seed germination and vegetative growth of Kachai Lemon (*Citrus jambhiri* Lush.) under low cost hydroponic condition" was conducted at Department of Horticulture, College of Agriculture, CAU, Imphal, Manipur during the year 2021-22. The experiment was laid out in Completely Randomized Design (CRD) with ten treatments replicated three times. Seeds were extracted from selected genotypes of Kachai Lemon which were collected from an orchard of Kachai Lemon, situated at Kachai Village, Ukhrul district, Manipur. A total of sixty seeds were taken for each treatment and were treated with the respective bio-fertilizers *viz*. Pseudomonas fluorescens, *Azotobacter*, *Azospirillum*, VAM, *Trichoderma viride* and their combinations. Total of 10 different treatments were formed i.e., T1- Pseudomonas fluorescens (5g/kg of seed), T-2- *Azotobacter* (5ml/kg of seed), T3- *Azospirillum* (5ml/kg of seed), T4- VAM (5ml/kg of seed), T5- *Trichoderma viride* (5ml/kg of seed), T6- Pseudomonas fluorescens (5g/kg of seed) + *Trichoderma viride* (5ml/kg of seed), T7- *Azotobacter* (5ml/kg of seed) + *Trichoderma viride* (5ml/kg of seed), T8- *Azospirillum* (5ml/kg of seed) + *Trichoderma viride* (5ml/kg of seed), T9- VAM (5ml/kg of seed) + *Trichoderma viride* (5ml/kg of seed) and T10- Water soaking (control) to study their effect on seed germination and vegetative growth parameters of Kachai Lemon. All the experimental seeds were uniformly maintained and irrigated during the whole period of investigation. At 4 months after sowing the seed germination and shoot and root growth parameters were recorded.

Germination vigour index (GVI) was computed using the formula given by Association of Official Seed Analysts (AOSA), 1983 [6].

$$GVI = \frac{x_1}{d_1} + \frac{x_2}{d_2} + \frac{x_3}{d_3} + \ldots + \frac{x_n}{d_n}$$

Where,

x1, x2, x3 …. xn are the number of seeds germinated and d1, d2, d3…. dn days taken for germination, respectively.

Seedling vigour index-I (SVI-I) and seedling vigour index-II (SVI-II) were calculated using the following formula given by Abdul-Baki and Anderson (1973) [3] and Bewley and Black (1982) [8].

SVI-I = Seedling length x Germination percentage

SVI-II = Seedling dry weight x Germination percentage

Results and Discussions

Days taken for 50% and complete germination

From the data given in Table 1, a statistically significant differences were found among the different bio-fertilizer treatments and their combined effect in terms of days taken for 50% and complete germination. Minimum days taken for 50% germination (62.43 days) and complete germination (87.09 days) of seeds were recorded in the treatment T_7-*Azotobacter* followed by 63.31 and 88.24 days for 50% and complete germination, respectively in the treatment T_5-*Trichoderma viride*. *Azotobacter* is known for excretion of phytohormones such as auxin and gibberellin, etc., that aid in early development and seed germination. *Trichoderma* spp. is also known to promote plant growth and limits the growth of plant pathogens (Siddiquee, 2014) [35]. Similar findings were reported by Nainwad *et al.* (2018) [25] in Rangpur lime, Geethanjali *et al*. (2021) [16] in aonla, Altomare *et al.* (1999) [4] in papaya and Rabari *et al*. (2019) [29] in bottle gourd.

Seed germination percentage (%)

The data obtained from Table 1 revealed that the treatment T7 -*Azotobacter* + *Trichoderma viride* delivered the highest seed germination percentage (91.67%), which was significantly superior among all the other treatments and it was followed by T5 -*Trichoderma viride* with mean 90%. *Azotobacter* fixes extra nitrogen from the atmosphere and produces growth promoting materials like auxin, cytokinin, etc. which might have resulted in consequent enhancement in the metabolic activities resulting in higher germination. Pathak *et al*. (2013) [27] and Nainwad *et al*. (2018) [25] also recorded the positive effect of *Azotobacter* on highest seed germination percentage in guava and Rangpur lime, respectively. Additionally, *Trichoderma viride* has its antagonistic effect against the phytopathogens that protects the inoculated seeds from fungal infection and finally leads to enhancement of seed germination (Doni *et al*., 2014) [15]. This finding is in conformity with the results of Raman (2012) [30] who reported that the highest seed germination percentage of apple was obtained from the combination of *Azotobacter* chroococcum and *Trichoderma viride* over all the treatments.

Germination vigour index (GVI)

Among the various treatments, T_7-*Azotobacter* + *Trichoderma viride* recorded the highest germination vigour index (0.63) which was found non-significant difference with T_5 (0.61), T_3 (0.59), T_8 (0.54), T_4 (0.53), T_1 (0.48), T_2 (0.42) and T_{10} (0.40). This might be due to high concentration of nutrients especially nitrogen and other phytohormones along with the production of secondary metabolites and antibiotics that facilitates plant growth and development. According to the findings of Mukhtar (2008) [23], germination index was significantly increased in seed treatment with *Trichoderma* spp. as compared to control. Similarly, highest germination index (1.88) was recorded in the treatment combination of castor cake + *Trichoderma harzianum* + *Arbuscular mycorrhiza* fungi by Devi *et al*. (2019) [13] in papaya.

Fungal infection percentage in germination stage (%)

Data pertaining to Table 1 showed that the minimum fungal incidence was observed in T_5 -*Trichoderma viride* which was superior among all other treatments with mean value 1.67%, followed by T_7 & T_9 with the same mean (3.33%). The

secondary metabolites secreted by *Trichoderma* spp. have proven its role in suppressing the growth of pathogenic microorganisms and stimulating the plant growth (Kullnig *et al*., 2000; Kubicek *et al*., 2001; Zin and Badaluddin, 2020) [20, 19, 43]. Antibiosis, micoparasitism and competition are the main features through which *Trichoderma* spp. react to the presence of other competitive pathogens, thereby preventing and obstructing their development (Sood *et al*., 2020) [39]. Similarly, Awad *et al*. (2018) [7] also reported the mechanism of *Trichoderma viride* that inhibits the mycelial growth of parasites. This result is in line with the findings of Nasratullah (2020) [24] in Rangpur Lime with the minimum fungal incidence (0.08%) in *Trichoderma viride* inoculated seeds under hydroponic condition.

Table 1: Effect of different bio-fertilizers on days taken for 50% germination, complete germination, seed germination%, germination vigour index and fungal infection% in germination stage of Kachai Lemon under hydroponic condition

Treatments	Days for 50% germination	Days for complete germination	Seed germination%	GVI	Fungal infection% in germination stage
T_1 -*Pseudomonas fluorescens* (5 g/kg)	70.99^{ef}	107.82^{g}	86.67^{d}	0.48^{b}	20.00^{h}
T_2 -*Azotobacter* (5 ml/kg)	72.14^{f}	105.44^{f}	81.67^{b}	0.47^{b}	16.67^{g}
T_3 -*Azospirillum* (5 ml/kg)	66.44^{bc}	88.99^{ab}	88.33^{e}	0.59^{d}	11.67^{f}
T_4 -VAM (5 ml/kg)	68.92^{cde}	97.59^{d}	86.67^{d}	0.53^{c}	8.33^{c}
T_5 -*Trichoderma viride* (5ml/kg)	63.31^{a}	88.24^{ab}	90.00^{f}	0.61^{de}	1.67^{a}
T_6 -*Pseudomonas fluorescens* (5 g/kg) + *Trichoderma viride* (5 ml/kg)	70.11^{def}	100.02^{e}	88.33^{e}	0.53^{c}	5.00^{c}
T_7 -*Azotobacter* (5 ml/kg) + *Trichoderma viride* (5 ml/kg)	62.43^{a}	87.09^{a}	91.67^{g}	0.63^{e}	3.33^{b}
T_8 -*Azospirillum* (5 ml/kg) + *Trichoderma viride* (5 ml/kg)	67.81^{bcd}	93.21^{c}	83.33^{c}	0.54^{c}	6.67^{d}
T_9 -VAM (5 ml/kg) + *Trichoderma viride* (5 ml/kg)	64.96^{ab}	89.41^{b}	88.33^{e}	0.59^{d}	3.33^{b}
T_{10} -Water soaking (control)	80.29^{g}	113.45^{h}	76.66^{a}	0.40^{a}	68.33^{i}
S.Em ±	1.02	0.77	0.05	0.01	0.46
CD @ 5%	3.03	2.27	0.15	0.02	1.36

NB: GVI*-Germination vigour index

The superscript letter signifies that the treatment means with similar letters are at par at 5% level of significance, while the means with different letters are significantly different at 5% level of significance. These letters have been affixed based on CD-value comparison of treatment means.

Plant height, leaf area/plant and root length

As regards with different bio-fertilizers, significant differences were noted between the treatments for plant height under the present investigation. Table 2 indicates that the seeds sown under the treatment T7 -*Azotobacter* + *Trichoderma viride* recorded the highest plant height (7.63 cm) after 4 months of sowing which was non-significantly different with T8 (7.47 cm), T5 (7.43 cm) and T2 & T3 (7.37 cm) respectively. Similarly, the treatments T7 -*Azotobacter* + *Trichoderma viride* and T8 -*Azospirillum* + *Trichoderma viride* delivered the maximum leaf area per plant having the same mean value of 2.10 cm2, followed by T5 -*Trichoderma viride* (2.03 cm2) and T2 -*Azotobacter* (1.88 cm^2). Furthermore, the treatments T9, T1, T6 and T3 having the leaf area per plant 1.47 cm^2, 1.50 cm^2, 1.52 cm^2 and 1.82 cm^2 respectively were statistically at par with each other at 5% level of significance. The statistical analysis of Table 2 showed that the different bio-fertilizer combinations significantly affected the root length of the saplings after 4 months of sowing. Among the various treatments, the maximum root length (8.97 cm) was recorded in T7-*Azotobacter* + *Trichoderma viride*, which was found statistically at par with the mean root length registered in T2, T3, T5, T6 and T8 (8.40, 8.60, 8.77, 8.33 and 8.80 cm, respectively).

Azotobacter spp. and *Azospirillum* spp. promote root growth as they are efficient nitrogen fixers (Borda-Molina *et al*., 2009; Rueda *et al*., 2016) [10, 31] and synthesize growth substances like auxin, cytokinin and GA like substances which greatly influence plant growth, resulting in improvements in uptake of nutrients and water. Prabhakar (2013) [28] has reported that seed inoculation with nitrogen fixing micro-organisms including *Azotobacter*, *Azospirillum* and Trichoderma spp. significantly increased the plumule and radicle length and reduced the attack of pathogens. Similarly, Bhadauria *et al*. (2000) [9] and Parameswari *et al*. (2001) [26] observed the significant increase in shoot length and root length of aonla seedlings and tamarind respectively with the inoculation of *Azospirillum*. Rangpur Lime seed inoculated with *Trichoderma viride* yielded the maximum plant height (15.67 cm) under hydroponic condition (Nasratullah, 2020) [24]. Similarly, Raman (2012) [30] reported that maximum shoot growth was observed on inoculation of apple seeds with *Azotobacter* chroococcum + Pseudomonas striata + *Trichoderma viride*. There was significant increase in leaf area and root length of strawberry in hydroponic system inoculated with *Azotobacter* and *Azospirillum* separately when combined with three nitrogen levels (50, 100 and 150 ppm) (Rueda *et al*., 2016) [31]. This is in agreement with the results obtained by Umar *et al*. (2009) [41] that better results were obtained when consortium of *Azotobacter* and nitrogen fertilization were used.

Seedling vigour index-I (SVI-I) and seedling vigour index-II (SVI-II)

From the Table 3, the data recorded after 4 months of sowing signify that the different treatments have significant impact on the SVI-II and SVI-II. Among the various treatments, the maximum SVI-I (1522) and SVI-II (17.33) were recorded under the treatment T7 -*Azotobacter* + *Trichoderma viride*, which was significantly superior among all other treatments, followed by T5 -*Trichoderma viride* having mean SVI-I and SVI-II of 1458.30 and 15.30, respectively. Doni *et al*. (2014) [15] concluded that Trichoderma spp. is able to enhance seed germination and vigour. This result is in tune with the findings of Lay *et al*. (2013) [22] in papaya and Lanjhiyana *et al*. (2020) [21] in papaya where significantly higher SVI-I and SVI-II were reported under the seed treatment of papaya with *Azotobacter* chroococcum.

Table 2: Effect of different bio-fertilizers on plant height (cm), leaf area (cm^2), root length (cm) SVI-I and SVI-II of Kachai Lemon saplings after 4 months of sowing under hydroponic condition

Treatments	Plant height (cm)	Leaf area/plant (cm^2)	Root length (cm)	SVI-I	SVI-II
T_1 - *Pseudomonas fluorescens* (5 g/kg)	6.87^{bc}	1.50^{ab}	7.83^{abc}	$1,273.80^{c}$	10.75^{c}
T_2 - *Azotobacter* (5 ml/kg)	7.37^{de}	1.88^{cd}	8.40^{cde}	$1,287.70^{d}$	10.86^{c}
T_3 - *Azospirillum* (5 ml/kg)	7.37^{de}	1.82^{bcd}	8.60^{de}	$1,410.40^{f}$	10.51^{bc}
T_4 - VAM (5 ml/kg)	6.00^{a}	1.33^{a}	7.43^{ab}	$1,164.00^{b}$	9.71^{b}
T_5 - *Trichoderma viride* (5 ml/kg)	7.43^{de}	2.03^{d}	8.77^{de}	$1,458.30^{g}$	15.30^{f}
T_6 - *Pseudomonas fluorescens* (5 g/kg) + *Trichoderma viride* (5 ml/kg)	7.10^{cd}	1.52^{abc}	8.33^{cde}	$1,362.90^{e}$	14.22^{e}
T_7 - *Azotobacter* (5 ml/kg) + *Trichoderma viride* (5 ml/kg)	7.63^{e}	2.10^{d}	8.97^{e}	$1,522.00^{h}$	17.33^{g}
T_8 - *Azospirillum* (5 ml/kg) + *Trichoderma viride* (5ml/kg)	7.47^{de}	2.10^{d}	8.80^{de}	$1,355.50^{e}$	12.58^{d}
T_9 - VAM (5 ml/kg) + *Trichoderma viride* (5 ml/kg)	6.57^{b}	1.47^{ab}	8.10^{bcd}	$1,295.50^{d}$	12.63^{d}
T_{10} - Water soaking (control)	5.53^{a}	1.30^{a}	7.10^{a}	968.40^{a}	8.43^{a}
S.Em±	0.16	0.12	0.25	3.60	0.33
CD @ 5%	0.48	0.36	0.74	10.69	0.97

NB: SVI-I-Seedling vigour index-I, SVI-II-Seedling vigour index-II

The superscript letter signifies that the treatment means with similar letters are at par at 5% level of significance, while the means with different letters are significantly different at 5% level of significance. These letters have been affixed based on CD-value comparison of treatment means.

Fresh weight and dry weight of shoot and root

According to the data tabulated in Table 3, the highest shoot (0.240 g) and root (0.054 g) fresh weight were noted under the same treatment T_7 -*Azotobacter* + *Trichoderma viride* followed by T_5 -*Trichoderma viride* with the mean shoot fresh weight 0.223 g and mean root fresh weight 0.051 g. Similar trend was also observed that the same treatment T_7-*Azotobacter* + *Trichoderma viride* yielded the maximum dry weight of shoot (0.163 g) and root (0.026 g), followed by T_5 - *Trichoderma viride* with the mean shoot dry weight and root dry weight of 0.145 and 0,025 g, respectively. This may be due to increase in overall growth of shoot and root in T_7 which leads to overall assimilation and redistribution of food material with the saplings. *Azotobacter* is an efficient nitrogen fixer that promotes root growth and also responsible for an increase in dry matter (Borda-Molina *et al*., 2009) [10]. It is reported that increased production of IAA correlates with the increased dry matter production (Shanmugaiah *et al*., 2009) [33]. This is in concordance to the findings of Raman (2012) [30], who had also reported a significant increase in biomass of apple seedlings on fresh weight basis in treatment combination of *Azotobacter chrooccocum* + *Pseudomonas striata* + *Trichoderma viride*. Yadav *et al*. (2012) also discussed the effect of *Azotobacter* in yielding the better result of dry weight of acid lime seedlings under the medium combination soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with *Azotobacter*. The present finding corroborates the findings of Velmourougane *et al*. (2017) [42] in chickpea and Rueda *et al*. (2016) [31] in strawberry.

Table 3: Effect of different bio-fertilizers on fresh weight and dry weight of shoot and root of Kachai Lemon after 4 months of sowing under hydroponic condition

Treatments	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
T_1 -*Pseudomonas fluorescens* (5g/kg)	0.187^{c}	0.034^{ab}	0.106^{b}	0.018^{a}
T_2 -*Azotobacter* (5ml/kg)	0.187^{c}	0.040^{c}	0.113^{b}	0.020^{ab}
T_3 -*Azospirillum* (5ml/kg)	0.157^{b}	0.044^{d}	0.098^{a}	0.021^{abc}
T_4 -VAM (5ml/kg)	0.160^{b}	0.036^{b}	0.094^{a}	0.019^{ab}
T_5 -*Trichoderma viride* (5ml/kg)	0.223^{d}	0.051^{fg}	0.145^{d}	0.025^{cd}
T_6 -*Pseudomonas fluorescens* (5g/kg) + *Trichoderma viride* (5ml/kg)	0.203^{c}	0.048^{ef}	0.138^{d}	0.023^{bc}
T_7 -*Azotobacter* (5ml/kg) + *Trichoderma viride* (5ml/kg)	0.240^{d}	0.054^{g}	0.163^{e}	0.026^{d}
T_8 -*Azospirillum* (5ml/kg) + *Trichoderma viride* (5ml/kg)	0.193^{c}	0.045^{de}	0.128^{c}	0.023^{bc}
T_9 -VAM (5ml/kg) + *Trichoderma viride* (5ml/kg)	0.193^{c}	0.036^{b}	0.123^{c}	0.020^{ab}
T_{10} -Water soaking (control)	0.137^{a}	0.032^{a}	0.093^{a}	0.017^{a}
S. Em±	0.006	0.001	0.002	0.001
CD @ 5%	0.019	0.003	0.007	0.004

Conclusion

From the present experimental findings, it can be concluded that pre-sowing seed treatment Kachai Lemon with different bio-fertilizer treatments had significant effect on seed germination and various shoot and root parameters. Among the different bio-fertilizer treatments, it was revealed that, treatment combination of *Azotobacter* + *Trichoderma viride* (T7) reduced days taken for 50% and complete germination of seeds, enhanced seed germination percentage, germination vigour index (GVI), plant height, leaf area/plant, root length, seedling vigour index-I, seedling vigour index-II, fresh weight and dry weight of shoot and root. Finally, it can be concluded that application of T7-*Azotobacter* + *Trichoderma viride* showing the best results among all the treatments may be recommended for the enhancement of seed germination and successful growth and development of Kachai Lemon saplings.

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