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Potential of Endophytic Fungal Isolates in Improving Productivity and Postharvest Marketable Fruit Qualities of Tomato

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Abstract

In Tanzania, low tomato (*Solanum lycopersicum*) yield and postharvest marketable fruit qualities remain significant limitations for tomato grower's profit. Seven endophytic fungal isolates from pyrethrum and Lemongrass leaf and or flowers were evaluated to promote tomato productivity and fruit qualities. The isolates were inoculated by priming the seeds in spore suspension, and the variations in the number of germinated seeds, seedling vigour, yield and fruit qualities were compared across the treatments. It was observed that only three isolates induced more than 50% seed germination. Epf1 and Epl1 isolates showed significant promotion of seed germination ($F_{0.05\ (pdf,\ 23)} = 8.121$ and $P = 0.001$) and seedling vigour. Isolate Epf1 increased fruit number (18.4%) and weight (17%) but reduced fruit size (0.63%); the Epl1 increased fruit size (12%) and reduced fruit number (14.4%). The Epf1 induced delayed fruit ripening, colour development and softening, decaying, and prolonged shelf life by 9 days. These findings indicate that promoting tomato productivity and postharvest marketable fruit qualities is isolate-specific; therefore, it requires diligent screening. Incorporation of the Epf1 isolate through seed priming with fungal spore suspension demonstrated a high potential for increasing tomato productivity and postharvest marketable fruit qualities.

Keywords: Fungal Endophytes; tomato productivity; postharvest fruit qualities; mycotoxins contamination

Introduction

Tomatoes are in high demand worldwide due to their important nutritional content and commercial significance. In Tanzania, tomato production increased from 30,000 tons in 1971 to 463,987 tons in 2020, thus growing at an average annual rate of 6% (FAOSTAT 2020). The average tomato yield ranges from 2.2 to 3.3 t/ha, 12% of the world's average productivity of 27.5 t/ha. Tanzania started to export tomatoes to neighbouring countries, including Kenya, DRC Congo, Zambia, Comoro, the Middle East and Europe (Mutayoba and Ngaruko 2015) nearly three decades ago. However, the tomato production and export levels are not stable. They are far below the more efficient producers (Mutayoba and Ngaruko 2018) who continue to expand their exports while maintaining the required market standards.

The potential for increasing the production of vegetables in Tanzania is enormous, but realising the profits requires increased production efficiency using modern inputs and technologies. Technology based on the use of microbes in promoting healthy plant growth and development has gained popularity in the agricultural industry. Microbes living inside the tissues of higher plants, especially the endophytic fungi, have attracted the attention of many researchers due to their rich diversity and exceptional

ability to produce novel and complex bioactive metabolites (Strobel 2018). Fungal endophytes from the genus *Fusarium sp*. have demonstrated great agricultural importance not only by acting as biocontrol agents (de Lamo et al. 2018, Constantin et al. 2019) but also by producing fungicidal and nematicidal chemicals. A recent study by Morsy et al. (2020) on the use of fungal endophytes to promote plant growth or increase crop tolerance to abiotic stress in tomatoes showed an increased yield of symbiotic tomatoes compared to non-symbiotic ones. Kumar and Kaushik (2013) suspected the presence of specific secondary metabolites that elicit the interactions of growth-promoting substances for plant healthy growth but also increased crop yields and adaptation to biotic and abiotic stresses (Rodriguez et al. 2009). However, apart from the production of useful secondary metabolites that can effectively defend the host plant and promote effective nutrient uptake, like other fungi, endophytes of the genus *Fusarium sp*. sometimes produce toxic metabolites (mycotoxins) of potentially serious health complications in humans and animals. This study, therefore, evaluated three out of seven screened endophytic fungal isolates colonizing pyrethrum and lemongrass leaf and or flowers for promotion of tomato productivity and postharvest marketable fruit qualities and screened for potential contamination of mycotoxins in harvested tomato fruits.

Materials and Methods

Study site

The study was conducted at Tengeru Horticulture Research Institute farm (36º58´ and 37º00´E; 03º37´ and 03º58´ S: 1290 m a.s.l). Seven fungal endophyte isolates were inoculated into tomato seeds, and resultant plants (symbiotic plants) were grown and observed in the screen house. The evaluation of postharvest marketable fruit qualities and experiments for detection and analysis of mycotoxins was conducted at the Nelson Mandela Institute of Science and Technology laboratory - Arusha.

Screening for endophytic fungal isolates-compatibility on tomato seed germination and seedling vigor

The blotter method was used to screen seven endophytic fungal isolates' effects on tomato seed germination and seedling vigour. Sixty tomato seeds of the Tanya variety obtained from Tengeru Horticultural Institute were primed using spore suspension prepared from pure endophytic isolates. The isolates were obtained by culturing sterilised small pieces (approximately 5 m x 10 mm) of pyrethrum leaf (Epl1, Epl2), flowers (Epf1, Epf2, Epf3) and lemongrass leaf (Elg1, Egl2) samples on potatoes dextrose agar (PDA) culture media incubated at 27 ºC for 7 days. Thereafter, a single spore was subcultured to form a pure fungi culture. The spore suspensions were made from 10-day-old cultures of each isolate grown on PDA, in which 5 ml of sterilized distilled water was added to the culture, and the spores were dislodged using a sterilized glass spreader. The resulting spore suspensions were transferred into sterilized petri dishes, and spore concentrations were adjusted to 1×106 spores/ml using a Hemocytometer, according to Wijesooriya and Deshappriya (2016). The control treatment was prepared by soaking the tomato seeds in sterilized distilled water overnight (24 hours), according to Lalngaihawmi et al. (2018). The treated seeds were then transferred into a new Petri dish containing wet blotter paper and incubated at room temperature 28 ± 2°C for 15 days. The blotters were periodically re-wetted to prevent drying and observation was done at intervals of 3 days for the period of 15 days. The seed germination rate was determined as the number of seeds germinated at specific time intervals (after every 3 days). Percent germination was calculated on the 15th day using the following formula:

$$\text{Percent germination} = \frac{(\text{Number of seeds geminated})}{(\text{Total number of seeds})} \times 100$$

Thirty (30) healthily germinated tomato seeds from Epl1, Epf1 and Elg1 treatments were transferred into sterilized plastic pots of 10 L capacity half-filled with sterilized soil for seedling development. Each treatment consisted of ten pots with three healthily germinated seeds, which were later trimmed to retain only one healthy seedling per pot. Other treatments: Epl2, Epf2, Epf3, and Elg2 produced no or very few germinated seeds (less than 50%); therefore, they were considered not compatible with tomatoes. The pots were then transferred into the screen house and arranged in a completely randomized design with three replications. The growth promotion effects of the endophytic isolates on tomato seedlings were assessed in terms of seedling vigour and seedling height within six (6) weeks.

Evaluation of the effects of endophytic fungal isolates on tomato productivity

A total of 800 tomato seeds (200 seeds for each treatment, including the control) of the variety Tanya were used during yield evaluation. The seeds were rinsed in distilled water and left to dry, then soaked overnight in spore suspension of each pure isolate with adjusted spore concentrations. The control treatment was prepared by soaking the seeds in distilled water overnight. The pretreated seeds were transferred into germination trays and left to germinate and grow in a nursery for thirty days. Tomato plants grown from tomato seeds treated with endophytic fungal isolates (Epf1, Epl1, and Elg1) were regarded as symbiotic plants (colonized by endophytic fungal) and plants grown from seeds treated with distilled water were considered as non-symbiotic plants.

Ninety tomato seedlings (30 Days old) of nearly uniform height and thickness from each treatment were selected and transplanted in the screen house. The treatments were arranged in a randomized complete block design in four plots i.e. one for each treatment randomly replicated on an area of 17 m x 14 m. Each block of 17 m × 3 m consisted of four plots. Each plot consisted of two parallel rows of 15 plants, each spaced 50 by 50 cm between plants and rows and 100 cm between adjacent plots. The treatments were replicated three times. The crop management procedures and conditions were adhered to and optimized. The data on number of fruits produced, fruit size, and fruit weight were recorded and analyzed as done by Litsingera et al. (2011).

Evaluation of the effects of endophytic fungal isolates on Postharvest marketable fruit qualities

The postharvest marketable fruit quality was assessed based on observable changes on 1 kg of uniform-sized tomato fruits harvested from each treatment. The assessed components included changes in fruit: colour, firmness/softness, weight, moisture content, dry matter content, disease or decay incidences, and the overall tomato shelf life. The treatments were arranged in a completely randomized design (CRD) in three replications. The numerical scale of 1–5, (where 1 = turning, 2 = pink, 3 = light red, 4 = red, 5 = ripe red) was used to score changes in tomato colour as described by Wills et al. (1981), and a scale of 1-6 (where 1 = hard, 2 = sprung, 3 = between sprung and eating ripe, 4 = eating ripe, 5 = overripe, and 6 = rotten) was used to score the degree of fruit firmness/softness. The change in tomato fruit weight was calculated in terms of percent weight loss as described by Moneruzzaman (2009). The initial and final sample weights were recorded at the beginning of the trial and 16 days, respectively. The weight loss was calculated by the following formula:

$$\text{Percent weight loss} = \frac{(\text{Initial fruit weight} - \text{Final fruit weight})}{(\text{Initial fruit weight})} \times 100$$

Moisture content was obtained by grinding 100g of tomato fruits to make tomato pulp, followed by oven drying at 50 °C for 12 hours and 100 °C for 12 hours until a constant weight was attained. The oven-dried pulp was reweighed to obtain the final pulp weight. The moisture content of tomato pulp was calculated using the following formula:

$$\text{Percent Moisture Content} = \frac{(100\text{g-Final pulp weight})}{100\text{g}} \times 100$$

The per cent dry matter content of tomato pulp was estimated as a per cent deviation of the percent moisture content from 100%. The data for diseases or decaying incidence was determined by visual observations whereby 1 kg sample of ripened tomato fruits was washed thoroughly with distilled water and placed in a clean container and placed at room temperature (25 °C). The tomato fruits were checked after every 6 days for spots on the fruit's skin, texture softening and rotting signs and monitored for 30 days. The disease or decaying incidence was calculated using the following formula:

$$\text{Percent decaying incidence} = \frac{(\text{Number of decayed fruits})}{(\text{Total number of fruits at initial})} \times 100$$

The tomato fruit shelf life was calculated by counting a number of days required to maintain 50% of the tomato at fully ripe while retaining optimum marketing and eating qualities under storage conditions. The fruits were kept at room temperature under optimal relative humidity achieved by floor flooding, as described by Moneruzzaman (2009). The shelf-life data were recorded at 4-day intervals until 50% of the tomato in each treatment had lost the marketable qualities, i.e. 40 days for this experiment.

Determination of mycotoxin contaminations in harvested tomato fruits

The harvested tomato fruits were tested for trichothecenesT-2 and HT-2 produced by members of the genus *Fusarium sp.* using HPLC protocols. Mycotoxins produced by members of the Alternaria sp. were not tested due to the absence of legally established tolerances or limits for contamination in foods (Murphy et al. (2006)). The trichothecenes standards used were supplied from the Nelson Mandela Institute of Science and Technology biochemistry laboratory.

Sample preparations, Mycotoxin extraction, Clean up, and elution

Treatment samples of 1 kg each were washed thoroughly in distilled water and homogenized to allow even distribution of mycotoxins. Then, 10 g of the samples were loaded into a set of polypropylene centrifuge tubes (5cm3), followed by the addition of 2000 ml acetonitrile/methanol (40/60 v/v) and vortexed for 3 minutes. Column clean-up and extraction of trichothecenes were done by QuEChERS (quick, easy, cheap, effective, rugged, and safe) method without the SPE clean-up step, as done by Zhang et al. (2018). Extraction was achieved by shaking the sample at 3000 g for 3 minutes. After that, 1g of sodium chloride and 4 g of anhydrous magnesium sulfate were added to the mixture, followed by repeated shaking at 3000 g for 3 minutes to induce phase separation and mycotoxin partitioning. Subsequently, an aliquot of the supernatant layer measured 1mL was evaporated until dry, followed by pre-column derivatization of the trichothecenes. The residue was reconstituted in 900 μl of 10% acetonitrile. Then, 100μl of trifluoroacetic acid was added, and the mixture was incubated for 15 minutes at a temperature of 50°C. The derivatized solution was then centrifuged at 1000 g for 5 minutes. A mixture of Trichothecenes standard solutions (T-2 and HT-2) of the following concentrations 1ng/ml, 10ng/ml, 100ng/ml, 500ng/ml and 1000ng/ml was prepared using derivatizing reagent for calibration of trichothecenes curve.

Determination of mycotoxins

An aliquot of 20μl sample extract was injected into a high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) system equipped with a fluorescence detector type RF20A Shimadzu. The detection was carried out by a fluorescence detector with excitation of 365 nm and emission wavelengths of 450 nm with a total

run time of 15 minutes. The machine was set with mobile phase water, methanol, and acetonitrile in a ratio of 50:40:10, respectively. The column temperature was 50 °C with a 0.8 ml/minute flow rate. The method was validated according to SANCO/12571/ 2013, demonstrating the analytical performances' conformity with criteria established in regulation (EC) no. 178/2010 (Turner et al. 2009). The total mycotoxin (trichothecenes) on each sample was calculated using calibrations that were obtained from HPLC reading and dilution factors.

$$\text{Conc. of sample in ppb} = \frac{\text{conc in } \left(\frac{\text{ng}}{\text{ml}}\right) \times 1 \text{ ml} \times 100 \text{ ml} \times 2.5 \text{ dil. factor}}{4\text{ml} \times \text{weight of sample taken in g}}$$

Data analysis

One-way analysis of variance was used to test for the significant variation in isolate compatibility for effective symbiotic colonization of tomato plants through seed inoculation and the variation in seed germination, seedling vigour and tomato yields between symbiotic tomato plants and non-symbiotic plants across various endophytic fungal treatments. The variations of parameters for postharvest marketable fruit quality were expressed in percentages, and significant variation among treatments was established based on one-way ANOVA. Mycotoxin contamination levels detected by the HPLC analysis of tomato fruits harvested from symbiotic and non-symbiotic plants in various treatments were compared with data provided by "Food Code"(Codex Alimentarius) for each T-2 and HT-2 mycotoxins tested.

Results

Effects of endophytic fungal isolates on tomato seed germination

The results on the effects of endophytic fungal isolates on seed germination are presented in Table 1. It shows a significantly higher germination percent of tomato seeds treated with isolate Epl1 and Epf1. The two isolates also recorded relatively higher germination rates, with 12 to 23% of seeds germinating within the first 3 days compared to the germination rate of the control treatment (7%). On the other hand, isolate Elg1 attained 50% germination but had a significantly lower germination percentage and rate relative to the control. Other endophytic fungal isolates either did not induce germination at all (Epf2) or produced less than 50% germinated seeds (Epf3, Epl2 and Elg2), therefore, they were considered not compatible with tomato. These isolates displayed an overgrowth of fungal mycelia of black colour and stink smell, and the resultant seedlings were in poor health and died after being transferred to new ports. The analysis by one-way ANOVA on the isolate compatibility foreffective symbiotic colonization of tomato through seed priming revealed significant differences ($F_{0.05}$ (df, 39) = 8.121 and P = 0.001).

Table 1: Number of tomato seeds germinated following treated with different endophytic fungal isolates; presented in terms of mean germination and percent germination (N = 60)

Isolates	Mean Number of Germinates					% Germination
	Day 3	Day 6	Day 9	Day 12	Day 15	
Epf1	7±2.9	15±2.9	31±2.9	42 ±2.9	45±2.9[a]	75
Epl1	14±3.3	22±3.3	30±3.3	49±3.3	55±3.3[a]	91.7
Elg1	0±2.4	0±2.4	7±2.4	16±2.4	31±2.4[b]	51.7
Epf2	0	0	0	0	0[c]	0
Epl2	0±1.2	0±1.2	0±1.2	4±1.2	5±1.2[c]	8.3
Elg2	0±1.2	0±1.2	0±1.2	3±1.2	4±1.2[c]	6.7
Epf3	0 ±0.7	0 ±0.7	0 ±0.7	0±0.7	3±0.7[c]	5
Control	4±2.9	13±2.9	18±2.9	26±2.9	39±2.9[a]	65
P value					0.0001	
F value					8.121	

Tukey's (HSD) test; Means with same letter are not significantly different at P > 0.05

Effects of endophytic fungal isolates on tomato seedling growth

Seedlings of tomato seeds inoculated with a spore suspension of endophytic fungal isolates Epl1 and Epf1 showed significant promotion of seedling vigour (Plate 1) and accelerated seedling height (Figure 1) compared to the control. Isolate Epl1 also improved leaf colouration. Generally, endophytic fungal isolate Elg1 showed significant suppression of seedling growth and development compared to the control treatment. The analysis of the variation of the effects of the endophyte isolates on tomato seedling growth and development by one-way ANOVA revealed significant isolate influence ($F_{0.05}$ (df, 23) = 19.2 and P = 0.001).

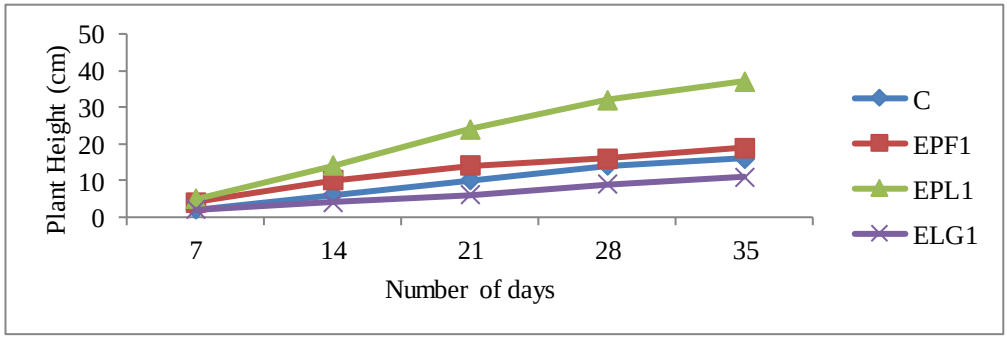

Figure 1: Variation in growth rates of symbiotic seedling (Epl1, Epf1 and Elg1) and non-symbiotic seedlings (control) expressed in terms of seedling height.

Plate 1: Variation of seedling vigor in response to different endophytic fungal isolate (Elg1 Epl1 and Epf1) treatments. Epl1, Epf1 and Elg1: symbiotic seedling plants of Epl1, Epf1 and Elg1 respectively

Effects of endophytic fungal isolates on tomato productivity

The results of the effects of endophyte isolates on tomato productivity are presented in Table 2. Symbiotic plants of Epf1 isolate produced a significantly higher number of fruits with higher average weight. The size of fruits produced by symbiotic plants was not different from the fruit size of the non-symbiotic plants ($P > 0.05$). Symbiotic plants of Epl1 produced fruits with significantly larger fruit sizes compared to other treatments. However, they produced fewer numbers of fruits and maintained the same fruit weight as those of the non-symbiotic plants. Symbiotic plants of Elg1 isolate produced a significantly lower number of fruits per plant of smaller sizes with low fruit weights. The analysis by one-way ANOVA showed significant isolate influence on number of fruits ($F_{0.05}$ (df, 15) = 28.1, P = 0.0001); fruit size ($F_{0.05}$ (df, 15) = 32, P = 0.0001); and fruit weight ($F_{0.05}$ (df, 15) = 91.9, P = 0.0001).

Table 2: Variation in number, size and weight of tomato fruits harvested from symbionts of fungal isolates and non-symbionts; presented as means and standard deviation (N = 30)

Isolates	Mean fruit number/ plant	Mean fruit size	Mean fruit weight /plants
Epl1	14.9±0.8 [b]	17.8±0.5 [a]	1.8±0.03 [b]
Epf1	20.6±1.8 [a]	15.8±0.4 [b]	2.1±0.12 [a]
Elg1	11.9±1.5 [c]	14.1±0.5 [c]	1.1±0.1 [c]
Control	17.4±1.1 [b]	15.9±0.7 [b]	1.8±0.03 [b]
P value	0.0001	0.0001	0.0001
F Value	28.1	32	91.9

Tukey's (HSD) test: Means with same letter are not significantly different at P> 0.05

Effects of endophytic fungal isolates on tomato postharvest marketable fruit qualities

The results on the effects of endophytic fungal isolates on the fruit ripening colour change, firmness or softness, decaying or disease incidences, weight reduction, change in moisture content, dry matter content and overall shelf life are summarized in Table 3. There was no significant variation in the tomato ripening colour score observed on the 16th day. However, tomato fruits harvested from Epf1 symbiotic and non-symbiotic plants exhibited delayed attainment of fully ripened red colour (12 days) compared to Epl1 and Elg1, which required 8 days. Tomato fruits from Epf1 symbiotic plants maintained an eating ripe state after 16 days,

while fruits from symbionts of Epl1 and Elg1isolates and the non-symbiotic plants were over ripened and rotten, respectively. One-way ANOVA showed significant variation of softening ($F_{0.05}$ (df19) = 3.84, p = 0.03) and Fruit decaying incidence ($F_{0.05}$ (df, 19) = 3.9, p = 0.02). Mean percentage weight and moisture content were significantly lower ($F_{0.05}$ (df, 15) = 106.6, p = 0.0001) in Epf1 symbionts and Epl1 respectively. The data on tomato shelf life was significantly high ($F_{0.05}$ (df, 15) = 78, p = 0.0001) on fruits harvested from symbiotic plants of isolate Epf1 and Epl1 and low on symbiotic plants of isolate Elg1.

Table 3: Variation of postharvest marketable fruit quality parameters of tomato fruits harvested from symbiotic and non-symbiotic plants of different endophytic isolates

Endophytic Isolates	Rating scale		Percentage change in tomato				Shelf Life
	Colour (1-5)	Softness (1-6)	Decaying	Weight	Moisture	Dry Matter	
Epf1	4.08 ^{a}	4.4 ^{b}	8.3 ^{b}	4.6 ^{a}	87.2 ^{b}	12.8 ^{b}	34 ^{a}
Epl1	4.86 ^{a}	5.36 ^{ab}	30 ^{ab}	7.5 ^{a}	76.1 ^{c}	23.9 ^{a}	28 ^{b}
Elg1	4.54 ^{a}	5.9 ^{a}	55.8 ^{a}	8.08 ^{a}	90.2 ^{a}	9.8 ^{c}	13 ^{c}
Control	4.14 ^{a}	5.04 ^{ab}	24.2 ^{ab}	5.8 ^{a}	86.4 ^{b}	13.6 ^{b}	25 ^{b}

Tukey's (HSD) test: Means with the same letter are not significantly different at $P > 0.05$

Potential for mycotoxin contaminations in harvested tomato fruits

The results of the assessment of potential Trichothesenes (T-2 and HT-2) contamination in harvested tomato fruits are presented in Figure 2. There was no single sample of tomato fruits contaminated with either Trichothesenes or other forms of mycotoxin (Figure 2).

Figure 2: Chromatogram of Trichothecenes free sample (A) and standard (B).

The endophytic fungal isolates in this study belong to members of the genus Fusarium sp and Alternaria sp, potential candidates for producing mycotoxins (Escrivá et al. 2017). Therefore, the harvested tomatoes were tested for trichothecenes (T-2 and HT-2) mycotoxins contaminations and the results showed no trace of the mycotoxins. Therefore, provides safety assurance in using endophytes Epf1 from pyrethrum for crop production.

Discussions

Influence of the endophytic fungal isolates on tomato seed germination and seedling growth

This study showed hastened and promoted tomato seed germination for at least two endophytic fungal isolates (Epl1 and Epf1) out of seven isolates (five from pyrethrum and two from Lemongrass) tested. Other isolates showed potent necrotrophic (Epf2), pathogenic (Epf3, Epl2 and Elg2) and suppressive (Elg1) effects on tomato seed. The sharp contrast in the germination percentage among the endophytic fungal isolates from the same source plant (pyrethrum and Lemongrass) emphasizes the considerable diversity of fungal endophytes and their potential utilization in the agricultural industry.

In this study, endophytic spore suspensions were used in the priming of tomato seeds to induce a symbiotic relationship with tomato plants. However, only three endophytic fungal isolates were compatible, successfully colonized the seeds, and incited varying degrees of tomato seed germination enhancements. These findings parallel that of Kucera et al. (2005), who reported the ability of endophytic fungi to secrete hydrolytic enzymes that could weaken the micropyle or hydrolyze the cell wall to allow early seed imbibition and hastens the germination process. Similarly, Hayat et al. (2020) demonstrated enhanced tomato seed germination through exogenous application of gibberellins (GA), ethylene and brassinosteroids (BR) phytohormones in different proportions. Though this study did not evaluate the factors responsible for the promotion of seed germination, the observed results on tomato seed germination by two endophytic fungi isolates, Epl1 and Epf1, suggest the presence of an intrinsic ability to secrete the germination-promoting factors.

Results in Figure 1 and Plate 1 showed clear variation in tomato seedling growth and development. Two isolates, Epl1 and Epf1, demonstrated significantly high tomato seedling vigor and height promotion. It was hypothesized that fungal spore inoculation of tomato seed led to symbiotic incorporation of endophytic fungal isolate into tomato plant. The observed ability of isolates Epl1 and Epf1 to promote seedling growth vigour and height without showing macroscopic symptoms of disease infections or nutrient deficiencies signifies an effective symbiosis establishment with the possible secretion of growth-promoting factors. Peguia and Valentino (2016) pointed out that endophytes that have established symbiotic relationships with their host plants secrete secondary metabolites that may induce a defence mechanism, enhance stimulating enzymes, or increase nutrient availability and uptake. Lalngaihawmi et al. (2018) reported maximum shoot length in symbiotic plants of endophytes *Cladosporium cladosporioides* due to the production of indole -3- acids, gibberellins and cytokinin growth-promoting substances. Similarly, symbiotic relations that produce biofertilizer effects have been reported by Kumar (2015) on Arbuscular mycorrhizae and some species of genus *Azospirillum* and *Trichoderma sp*. on rice plants. The fact that isolates Epl1 and Epf1 induced increased tomato seedling vigour and height could not be discounted. Still, the exact mechanism of the symbiotic action, whether the observed promotion was due to enhanced uptake of essential nutrients like N, P and Fe or adjusting hormone levels such as Auxins, cytokinins and gibberellins, was beyond the scope of this work. However, the findings clearly demonstrated that pyrethrum plants are a significant source of fungal endophytes of promising potential.

Influence of endophytic fungal isolates on tomato productivity and postharvest marketable fruit qualities

Results on tomato productivity indicated a significant positive influence of endophytic fungal isolates Epf1 and Epl1 on the mean number of fruits per plant, size, and weight. The influences were attributed to successfully establishing symbiotic associations with tomato plants following seed priming by endophyte spores. However, the endophytes' mode of growth and yield promotion were not studied. Studies associating the influence of endophytes on crop productivity with

endophyte's ability to produce growth-promoting factors or enhance enzymatic action that promotes nutrient uptake are quite evident (Rana et al., 2020). Contreras-Cornejo et al. (2011) reported the synthesis of the phytohormones related to auxin (indole-3-acetic acid, indole-3-acetaldehyde and indole-3-ethanol) by fungal species *Trichodermavirens* while working with *Arabidopsis thaliana*. Production of growth-promoting factors was also reported from three endophytic fungal strains of the genus Trichoderma sp, Fusarium sp, and Papulasporasp by Vitorino et al. (2016) while working with hybrids of *Eucalyptus grandis* and *Eucalyptus urophylla*. The fungal strains showed a direct positive influence on stem length, stem diameter, and fresh and dry biomass. This study reports a yield increase on symbiotic tomato plants of Epf1 by 18.4% for the number of fruits per plant, 17% mean fruit weight and on symbiotic tomato plants of Epl1 by 12% for mean fruit size. The two isolates belong to the genus *Fusarium* (Michael et al. 2020), suggesting the high possibility of having *an* intrinsic ability to synthesize phytohormones related to auxin.

Results on the effects of endophytic fungal isolates on tomato postharvest marketable fruit quality indicated significant variation whereby isolate Epf1 induced delay in the fruit ripening process, fruit colour change and prolonged the shelf life for nine more days compared to the control. A similar observation was reported by Su et al. (2015) while assessing the effects of the application of various hormone-like substances to "Mature-Green" tomato fruits. Treatment with ethylene and auxin hormones induced contrasting effects on tomato fruit colour. Treatment with the ethylene precursor amino cyclopropane carboxylic acid (ACC) significantly accelerated the transition from green to orange/red compared to controls. On the contrary, treatment with IAA induced a significant delay in the transition from green to orange/red compared to controls. After 96 h, IAA-treated fruits began to turn orange and never became red.

Colour change from green to red is a crucial indicator of tomato ripening. The change is associated with the degradation of chlorophylls and the shift of the carotenoid composition from leaf-like xanthophylls (mainly lutein and neoxanthin) to carotenes (mainly phytoene, lycopene and β-carotene) as described by Fraser et al. (1994). Therefore, suggest that IAA retards tomato ripening by affecting a set of key regulators, such as Rin, ethylene and ABA, and key effectors, such as genes for lycopene and β-xanthophyll biosynthesis and chlorophyll degradation. Though this study did not look at the mechanism of action or factors behind delayed ripening and prolonged shelf life, the effects exhibited by isolate Epf1 on fruit colour change, ripening, and shelf life are convincing enough to suggest the intrinsic ability to produce auxin-related phytohormones.

Conclusion and Recommendation

This work has established that some endophytic fungi from pyrethrum (*Chrysanthemum cinerariifolium*), a local medicinal plant, could artificially be adapted to other host plants and successfully produce symbiotic relationships. Three of seven endophytic fungi isolates evaluated promoted early seed germination and percent germination following seed priming with endophyte spores of pure endophyte isolates. Two endophytic fungal isolates, Epf1 and Epl1, increased seedling vigour, plant growth rates, tomato yield per plant and postharvest marketable fruit quality and shelf life. The improved yield aspects include increased fruit number and weight per plant and size. The assessment of mycotoxin contamination on tomatoes harvested from endophytic symbionts did not show any trace of mycotoxins. Therefore, endophyte isolates Epf1 and Epl1 could be incorporated into horticultural production to enhance tomato productivity and postharvest marketable fruit qualities.

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