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ENDOPHYTIC FUSARIUM SOLANI EXHIBITING POTENTIAL STRESS TOLERANCE AND ENHANCES GROWTH OF MAIZE IN SOUTH AFRICA

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ABSTRACT

New technological approaches harness the potential of endophytic fungi as growth promoters, utilizing their bioactive compounds to protect against herbivores, insects, and various unfavourable abiotic stresses, including temperature, salinity, drought, and heavy metals. *Zea mays* (L.) is considered as a staple food source in many countries including South Africa. Endophytic fungi are natural growth promoters without causing any disease or symptoms of diseases. In the current study, nine endophytic fungi were examined for abiotic stress tolerance and were inoculated in the maize seeds prior to plantation. The construction of the phylogenetic tree was based on consensus sequences (ITS) using endophytic fungi produced three clades with four subgroups. Among the 9 endophytic fungi, only 3 fungal isolates (END 15, MHE 55, RNK 4) grew on 3% and 6% salinity PDA. *Boeremia exigua* was the only isolate that could withstand 6%. *Fusarium solani* (MHE 55) survived at low pH. Furthermore, *Neurospora* sp. (GG 9) and *Fusarium solani* (MHE 55) grew at 37 ºC. Greenhouse experiments were conducted to establish the growth promoting properties. The highest plant height was observed in the treatment which was inoculated with *Fusarium oxysporum* (GG 8) followed by *Fusarium solani* (MHE 55), reaching 46 cm and 44 cm respectively. The significant enhancement in plant height, root weight, fresh weight and leaf size, demonstrate the potential use of the selected isolate as a growth promoter for maize. In contrast *Chaetomium* (PG 9) and *Alternaria* (MHE 68) displayed none to limited growth promoting properties. To the best of our knowledge, this is the first report on the potential use of *F. solani* isolated from South African geranium as a growth promoter. As the world is aiming at a more sustainable and eco-friendly agriculture, the use of such endophytes will contribute to better crop production and protection, hence more food availability.

Key words: *Fusarium solani*, Endophytes, Growth promotion, Maize, Stress tolerance, *Boeremia exigua, Chaetomium, Alternaria*

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INTRODUCTION

Fusarium solani is a well-known emerging opportunistic fungal pathogen in plants and immunocompromised patients. It is naturally occurring in the environment, commonly found in air, water and soil [1,2]. Furthermore, *Fusaria* are often referred to as soil-borne fungi which colonize the aerial plant parts thus becoming part of the normal mycoflora or act as plant pathogens on agricultural commodities. Agricultural plants may be infected by pathogenic *Fusaria* resulting in massive economic losses [3,4]. On the other hand, *Fusaria* can infect and colonize plants asymptomatically as endophytes [5]. Endophytic fungi harboured within the living tissues of plants play significant roles in plant growth and protection. Hence, these attributes play physiological and ecological roles in the plant [6]. Endophytes can either be fungi or bacteria that colonize and reside inside the healthy tissues of living plants without causing apparent harm/damage to the host. The roots, stems and leaves of plants are the main reservoirs of various endophytes [7]. Current research suggests that nearly all plant species harbour one or more endophytes [8], however only a very small proportion has been investigated among the 300,000 plant species worldwide [9]. Endophytic fungi are exceptional sources of bioactive natural compounds possessing antimicrobial properties; act as biofertilizer to enhance plant growth; and withstand biotic and abiotic stress conditions [10].

Zea mays (L.) is the botanical name for maize or corn that belongs to the family *Poaceae*. Maize has become a staple food to many African countries and other parts worldwide. Pathogenic infections by viruses, bacteria, nematodes, fungi, mycoplasmas and parasitic seed plants may possess a threat to the global food security [11]. Contamination of the maize plants can occur on the plantation site and during storage where kernels are subjected to infection by toxigenic fungi [12]. Dominant fungal pathogens are often *Aspergillus, Penicillium* and *Fusarium* that have the ability to produce aflatoxins, fumonisins and other mycotoxins. In addition, this will affect the quality of the grains, loss of product and risk to human and animal health [13]. Due to this, there may be a loss of quality seeds. Seed dressings are considered some of the best ways to reduce deterioration of seed in storage [14]. Treatments of seed is currently being used successfully in the field, the greenhouse and in storage. This study was intended to functionally characterise endophytes from selected medicinal plants *Sceletium tortuosum* L. (kanna) and *Pelargonium sidoides* (South African geranium) and assess their potential use as growth promoters of *Zea mays* (L.) individually in a greenhouse experiment by measuring plant height, root weight, fresh weight and leaf size. Results obtained were aimed at providing options for the use of these endophytes in the development of microbial formulations to enhance plant growth and *Zea*

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mays (L.) in particular, thus increasing crop productivity. To the best of our knowledge, this is the first report on endophytic fungi isolated from geranium plants which were used as growth promoters.

MATERIALS AND METHODS

Selection of Endophytic fungal isolates

Endophytic fungi were isolated from South African kanna and geranium medicinal plants. The rational for the selection of endophytic fungi that were utilized in the greenhouse experiment was based on previous data generated from the *in-vitro* antimicrobial activities and molecular analysis as described previously [15]. Table 1 shows a summary of all nine fungal isolates with their molecular identification, host plants and significant bioactive properties against pathogenic bacteria which were selected for the greenhouse experiments.

Stress tolerance assays

Abiotic stress tolerance assays (pH, salt and temperature) were conducted to determine resilience of endophytic fungi in unfavourable conditions. All growth media were supplemented with Kanamycin sulfate (50 µg/ml) and Chloramphenicol (50 µg/ml) to prevent growth of unwanted bacteria [16]. Salinity test was determined using PDA media supplemented with sodium chloride with different concentration (3%, 6%, 10% w/v) at 25 ºC for 2 weeks. The pH tolerance test was done adjusting in pH of Malt extract broth using hydrochloric acid (HCl, pH 2 and 3) and sodium hydroxide (NaOH, pH 12). Temperature tolerance test was examined by cultivating pure fresh fungal strains on PDA plates and incubated at different temperature conditions of 2 ºC, 25 ºC, 37 ºC and 50 ºC [17].

Phylogenetic analysis

Analyses of the internal transcribed spacer (ITS1 and ITS4) regions was conducted by Manganyi [15]. The ITS sequence data were utilized to construct phylogenetic trees (Maximum Likelihood method with 1000 bootstrap replication) using MEGA 7.0.21. Consensus sequences and alignment were accomplished by using BioEdit software 7.2.5 and results alignment was exported to Mega for tree construction.

Greenhouse trials

Fungal inoculum

Preserved component fungal cells were grown on Potato Dextrose agar (PDA) (Merck, Darmstadt, Germany) and aerobically incubated at 25 °C for 10 days. In order to achieve this, the mycelia of fungal isolates were inoculated into 250 mL Erlenmeyer flasks containing 50 mL of Malt Extract broth (MEB) (Merck, Darmstadt, Germany) and incubated aerobically while shaking on a rotary shaker (Labcon 3081U, Gauteng, South Africa) at 150 rpm for 5 days at 25 °C.The spore suspensions were withdrawn using sterile syringes and filtered through 0.25 mm,

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0.45 µm PALL Sterile Acrodisc Syringe Filters (Separations, South Africa) in order to remove fungal cell mass. The fungal extracts that may or may not possess secondary metabolites were transferred to clean sterile flasks and stored 4 °C in the cold room and used for further analysis [20].

Surface disinfection and Pre-treatment of maize seeds before planting

Zea mays (L.) seeds (BG5685B), acquired from Mpumalanga, Republic of South Africa's Pannar Seed Company, and were employed in the greenhouse studies using a conventional in vivo methodology. The maize seeds underwent chemical treatment to prevent diseases, which could have involved using a combination or singular application of antimicrobial, fungicidal, and insecticidal agents. Specifically, in this study, the maize seeds were immersed in a 1% solution of sodium hypochlorite (NaOCl) for 5 minutes followed by rinsing with distilled water. Due to this, the removal of chemical treatment and residues. The seeds and seedling trays were kept at room temperature for 24 hours to air dry.

Inoculation of the pasteurized soil

Spore suspensions of the endophytes were prepared by inoculating a plug of the fungal mycelia into 300 mL of Malt extract broth that was placed in a 500 mL beaker. The final concentration of fungal spore suspension in the broth was adjusted to 1 X 10^6 cfu/mL using a Neubauer heamocytometer. Artificial wounds were created in the seeds using a sterile scalpel blade and seeds were soaked in the fungal suspension for 48 hours. Negative control seeds were soaked in 300 mL of distilled water for 48 hours. Replication of five (5) seeds for each fungal extract were prepared and used in this experiment. The soil was autoclaved twice before transferring it into the trays and planting pots. Seeds were sowed on the trays and six days after sowing, the seedlings were transplanted into the planting pots [21].

Measurement of growth parameters

Plants were consistently monitored for signs of leaf chlorosis, browning of stem and leaves, wilting and cracking every 24 hours. The greenhouse experiments were terminated after four weeks. Growth parameters such plant height, root weight, leaf size and fresh weight were measured. For analysis, each experimental sample was prepared in triplicate. Data were subjected to analysis using (ANOVA) SPSS software.

RESULTS AND DISCUSSION

A total of nine endophytic fungi were isolated from two indigenous medicinal plants (*Sceletium tortuosum* and *Pelargonium sidoides*). The selection was based on several feature of the endophytic fungi listed in table 1. Molecular identification was previous conducted to confirm the scientific name of the fungal isolates.

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Stress tolerance assays

The abiotic stress tolerance was examined on the fungal isolates using their growth in different parameters of salt, pH and temperature. Among the 9 endophytic fungi, only 3 fungal isolates (MHE 55, RNK 4) were capable to withstand 3% and END 15 was able to grow at 6% salinity conditions. END 15, which was identified as *Boeremia exigua* var. pseudolilacis was the only isolate that could withstand 6%. *Fusarium solani* (MHE 55) was the only isolate that showed grow, at low pH (Table 2). All fungal isolates excelled in their ideal temperature of 25 ºC. In contrast, there was no growth at 50 ºC. However, *Neurospora* sp. (GG 9) and *Fusarium solani* (MHE 55) exhibited resistance at temperature of 37 ºC (Table 2). Ripa and colleagues [22] showed that endophytic fungi were able to tolerant abiotic stresses such as salt, heavy metals, drought, and temperature. Only two isolates (582PDA6 and 582PDA7) could withstand 2.5% salt concentration but not more than 5%. Other studies support that endophytic fungi aid in the adaptability of unfavourable abiotic stresses [17].

Phylogenetic tree

The sequence data was generated from previous study [15], however in this study, we compared the selected endophytic fungi using phylogenetic tree. The construction of the phylogenetic tree was based on consensus sequences (ITS) using endophytic fungi (Table 1) produced three clades with low bootstrap support. Group 1 formed the lowest bootstrap support of <50% while Group 2 has a higher bootstrap support shown at their respective nodes (Figure 1). All the investigated endophytic fungal isolates belonged to the phylum Ascomycota under different genera. The phylogenetic tree illustrated three distinct groups with several sub-clades. Overall resolution of the tree at genus and species level had a bootstrap support >50%. The first group was composed of four sequences, which clustered further into two sub-clades (1a, 1b). The second group involved four endophyte sequences, grouped into two sub-clades (2a, 2b). An interesting result was obtained from group 3 since *Fusarium solani* (MHE 55) was uncluttered even though there was another *Fusarium* (GG 8) in the tree. *Fusaria* (epiphytes and endophytes) are considered as one of the largest number of strains associated with agricultural productions. Depending on their role, they are capable of producing toxins or act as biological control agents in plants [23].

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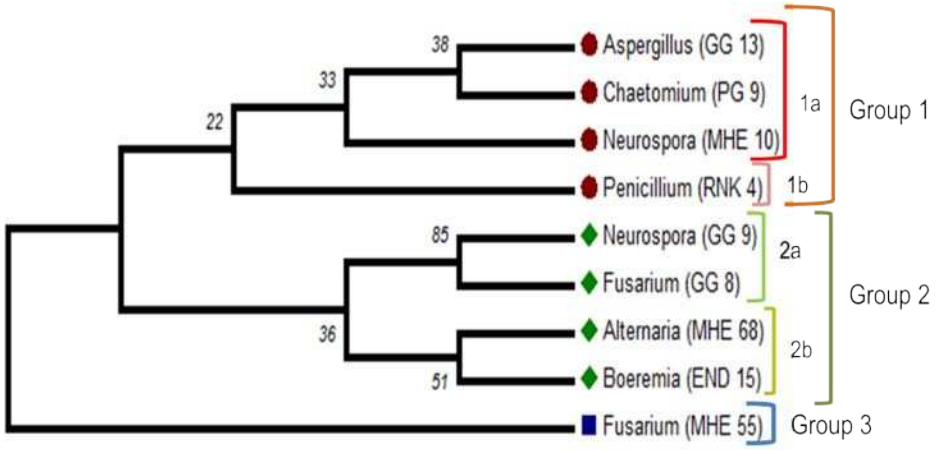

Figure 1: Phylogenetic tree constructed with sequences of the ITS regions of selected endophytic fungi using the Maximum Likelihood method with 1000 replicates

Weight of the seeds after inoculation

Table 3 listed the initial and final seed weight to calculate the moisture content (Equation 1). Extract GG 13 had the highest (58.4%) weight gained, followed by the control gaining 57.5%. Other extracts of significant weight gain were MHE 10 (49.6%), GG 8 (48.5%) and MHE 68 (48.1%).

$$\textbf{\% Weight increase} = \frac{Weight\ after - Weight\ before}{Weight\ before}\ x\ 100$$

Equation 1: General formula used to calculate the average weight gained
This is due to the absorption of moisture content within the seeds

Seed germination after inoculation

A success rate of 90% germination of seeds was observed in this trial. The control (water) seeds germinated faster and adequately when compared to the fungal treated seeds as is shown in Figure 2 (a and b). This may be due to the dense spore suspension and thick mycelia utilized in this study [24]. Furthermore, this could have influenced the low or non-geminated seeds, illustrated in Figure 2 (c) by blocking the incision portion. In this study, deionized water was used as a control as it was earlier reported to have the highest and fastest germination in *Ziziphus lotus* (L.) Lam shrub [24]. These results support our finding in this study. The thickness of the fungal extracts contributed greatly to the absorption of moisture by the seeds [24].

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Figure 2: Seed germination (a) control treatment (b) fungal treatments (c) low germinated seeds after 6 days

Effect of endophytic isolates on maize plant in the greenhouse (Growth parameters)

The growth parameters were checked every seven days until the 28th day after planting. The growth height for all treatments consistently increased with the plant age throughout the greenhouse experiments (Table 4). The highest average plant height by the twenty eighth day was observed in the treatment which was inoculated with *Fusarium oxysporum* (GG 8) followed by *Fusarium solani* (MHE 55), reaching 46 cm and 44 cm respectively. These were followed by GG 9 with average plant height of 42 cm then the control at 41 cm. These findings imply that some of the endophytic fungi employed in this investigation increased the height of the plants. The greenhouse experiments carried out by Machungo *et al.* [25] corroborate these conclusions employing banana plants in Uganda. Throughout the trials, endophytic F. The plant height of the oxysporum isolates (V5W2, Emb2.4o, and Eny7.11o) significantly increased by 11.3 %. Conversely however, the maize plants that were treated with *Chaetomium subaffine* (PG 8) extracts in the current study were the shortest plants (28 cm) as compared to the control. Hence, *Chaetomium subaffine* (PG 9) suppressed the growth of the maize plants.

Chaetomium subaffine (PG 9) extracts were noted to suppress the growth of maize plants, resulting in shorter plants compared to the control. In contrast to its suppressive effect on plant height, *Chaetomium subaffine* (PG 9) extracts resulted in a higher fresh weight compared to certain other extracts (weighing in at 5.8 g). This might seem contradictory to its impact on plant height but suggests that despite the suppressed height, it might have influenced other growth parameters like biomass accumulation or water content.

The highest fresh weight was *Boeremia* (END 15) and *Fusarium* (MHE 55) extracts by measuring an average of 11.7 g (100%) and 11.3 g (97%), respectively after 28 days. The lowest weight was observed with *Aspergillus* (GG 13) and *Chaetomium* (PG 9) extract, weighing in at 4.9 g (42%) and 5.8 g (50%) respectively. In another study, *Salvia miltiorrhiza* seedlings were inoculated with *Alternaria* sp. A13 and

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they exhibited significant increases in fresh weight, dry weight, and total phenolic acid [26]. In this study, the heaviest roots were observed with *Boeremia* (END 15), followed by *Fusarium* (MHE 55), *Fusarium* (GG 8) and *Alternaria* (MHE 68) treatment measuring 6.4 g (100%), 5.5 g (86%), 5.4 g (84%) and 5.2 g (81%), respectively. All growth parameters were illustrated and compared with each other. Inoculum (GG 13, PG 9, RNK 4) suppressed the growth of the maize plants represented in Figure 4.

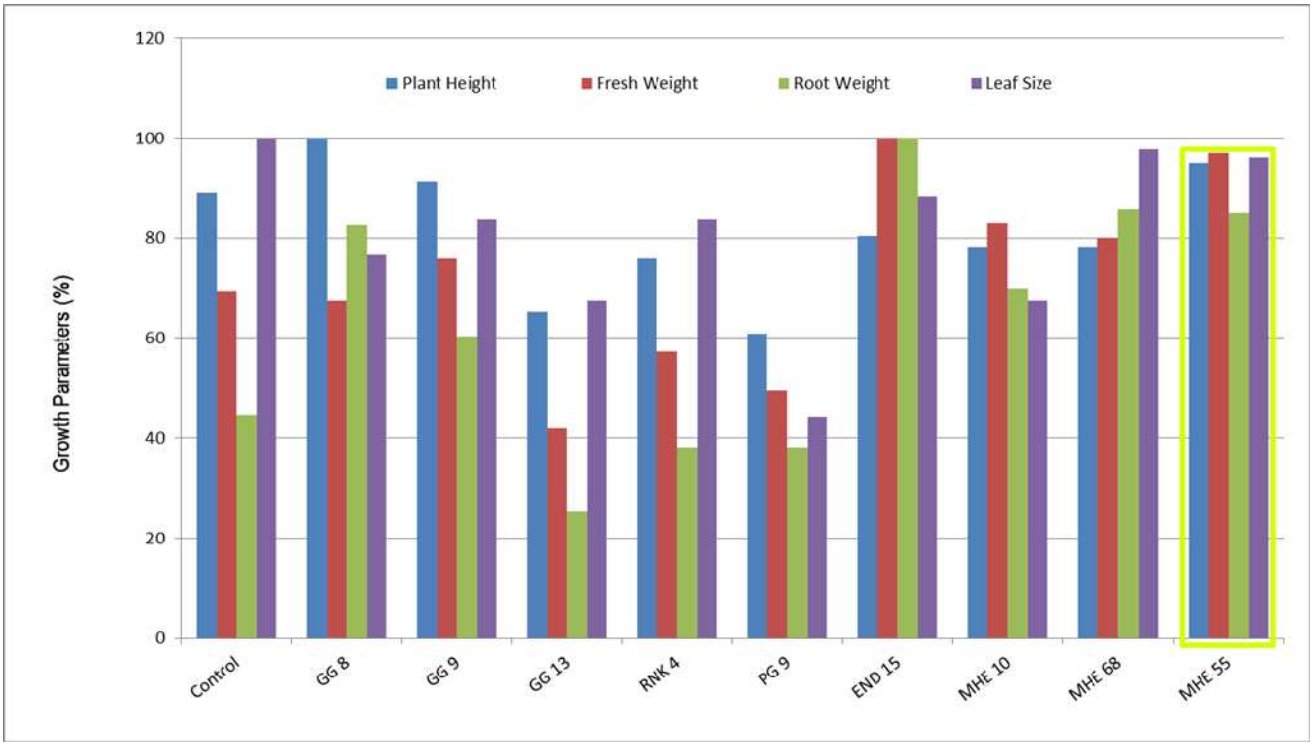

Figure 3: **Growth parameters of the fungal extracts analysed in maize plants after 28 days**

The largest leaves measured were from the control samples (43 cm). This was followed by *Fusarium* (MHE 55) having 42 cm. The smallest leaf was from the *Chaetomium* (PG 9) samples with 19 cm. The tallest plant was with *Fusarium* (GG 8) and the smallest plant inoculated by *Chaetomium* (PG 9) as shown in Figure 4.

Mutualistic relationship between the host plant and the endophytes have been associated with increasing population growth, health and regeneration [27]. Endophytic fungi colonize the plant and contribute to plant health, protection against herbivorous insects and plant pathogens. Therefore, the growth of the plant would be increased [28]. Several studies have reported *F. solani* as a plant pathogen in prominent agricultural commodities such as tomatoes [29], cucumber [30], and maize [31]. The findings in this study contradict the fact that *F. solani* is a well-

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known plant pathogen that is capable of producing mycotoxins. It is surprising that *F. solani* acts as a very effective growth promoter in maize.

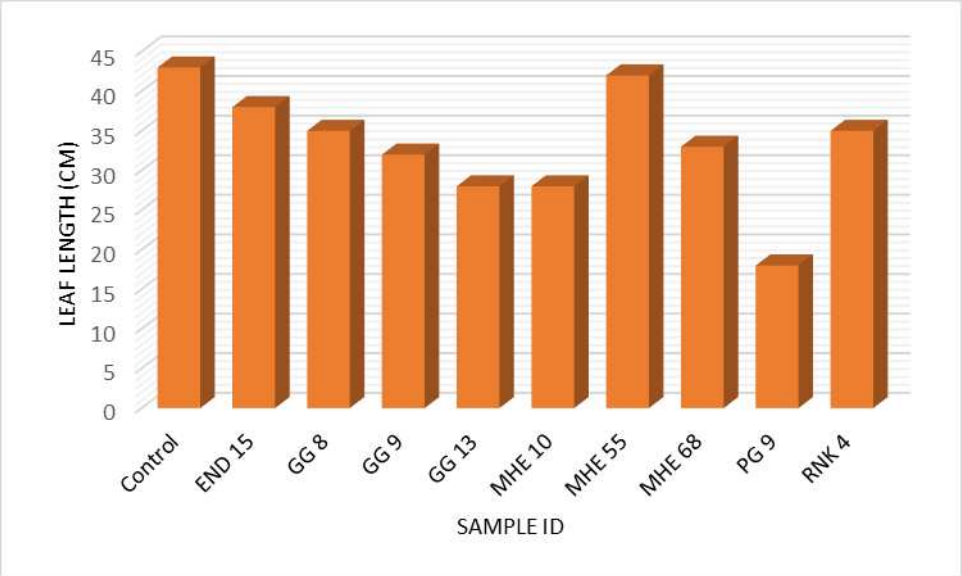

Figure 4: Average of leaf length after 28 days

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

In this study, endophytic *F. solani* (MHE 55) isolated from South African geranium plant exhibited significant growth promoting properties in maize. This provides an alternative approach to explore more sustainable and eco-friendly agriculture approach by discovering novel fungal extracts as growth promoters. The enhancement of plant height, fresh weight, root weight and leaf size were important parameters used for plant growth assessment in this study. It is also critical to evaluate the production of mycotoxin prior to inoculating the maize plants. These findings can be beneficial in the agricultural industry specifically as bio fertilisers. Hence, it will increase the production and help in the protection against unfavourable environments (abiotic stresses) resulting in more food availability. Endophytic fungi isolated from medicinal plants are good candidates for inoculum in agricultural crops as excellent growth promoters.

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DECLARATION OF COMPETING INTEREST

There is no conflict of interest.

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AUTHORS' CONTRIBUTIONS

M.C.M. was the main researcher, who was accountable for planning, conceptualised the project, interpretation of results, and edited the manuscript and executing the greenhouse experiments.

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Volume 24 No. 4
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Table 1: Summary of fungal strains used in the greenhouse experiment

Sample ID	Molecular ID	Host Plant	Antibacterial activity (mm)	Antifungal activity (mm)	References
END 15	*Boeremia exigua* var. *pseudolilacis*	Geranium *(P. sidoides)*	++	N/A	[18]
GG 8	*Fusarium oxysporum*	Kanna (*S. tortuosum* L.)	+	+	[19]
GG 9	*Neurospora sp.*	Kanna (*S. tortuosum* L.)	+	+	[19]
GG 13	*Aspergillus fumigatus*	Kanna (*S. tortuosum* L.)	+	+	[19]
MHE 10	*Neurospora crassa*	Geranium *(P. sidoides)*	+	N/A	[18]
MHE 55	*Fusarium solani*	Geranium *(P. sidoides)*	++	N/A	[18]
MHE 68	*Alternaria sp.*	Geranium *(P. sidoides)*	++	N/A	[18]
PG 9	*Chaetomium subaffine*	Geranium *(P. sidoides)*	+	N/A	[18]
RNK 4	*Penicillium glabrum*	Geranium *(P. sidoides)*	++	N/A	[18]

Table 2: Abiotic tolerance properties of the investigated endophytic fungi

Sample ID	Salinity (NaCl)			pH			Temperature			
	3%	6%	10%	2	3	12	2 ºC	25 ºC	37 ºC	50 ºC
END 15	++	+	-	-	-	-	-	+++	-	-
GG 8	-	-	-	-	-	-	-	+++	-	-
GG 9	-	-	-	-	-	-	-	+++	+	-
GG 13	-	-	-	-	-	-	-	+++	-	-
MHE 10	-	-	-	-	-	-	-	+++	-	-
MHE 55	+	-	-	+	+	-	-	+++	+	-
MHE 68	-	-	-	-	-	-	-	+++	-	-
PG 9	-	-	-	-	-	-	-	+++	-	-
RNK 4	+	-	-	-	-	-	-	+++	-	-

No growth, + slight growth; ++ moderate growth; +++ good growth

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Table 3: Average weight increase of five seeds before and after inoculation with the endophytic fungi

No.	Sample ID	Average Weight (g)	Weight after inoculation	Percentage of weight gained
1	END 15	1,576±0.10	2,291±0.02	45, 4%
2	GG 8	1,634±0.03	2,427±0.18	48,5%
3	GG 9	1,728±1.20	2,539±0.70	46.9%
4	GG 13	1,520±0.009	2,408±0.83	58,4%
5	MHE 10	1,636±1.00	2,448±0.00	49,6%
6	MHE 55	1,622±0.00	2,365±0.13	45.8%
7	MHE 68	1,558±0.50	2,308±0.20	48.1%
8	PG 9	1,636±0.12	2,348±0.02	43.5%
9	RNK 4	1,542±1.96	2,233±0.69	44.8%
10	Control	1, 532±0.25	2,413±0.09	57.5%

Data are means and standard deviation; ANOVA test (P > 0.05)

Table 4: Effect on endophytic fungi on the plant height (cm) over 28 days

Plant age (days)	Treatments (Plant height cm)									
	Control	END 15	GG 8	GG 9	GG 13	MHE 10	MHE 55	MHE 68	PG 9	RNK 4
7	11±0.24	8±0.04	13±0.83	10±0.02	6±0.11	9±0.40	13±0.33	8±1.00	9±0.01	9±0.30
14	19±0.11	17±0.01	25±0.25	23±1.70	14±0.24	17±0.13	25±0.18	17±0.12	15±0.35	18±0.24
21	33±0.26	29±1.37	34±1.05	34±0.85	21±0.34	26±0.00	35±1.02	25±0.45	22±1.06	26±0.05
28	41±1.01	37±0.31	46±0.00	42±0.01	30±0.05	36±0.84	44±0.67	36±0.02	28±0.12	35±2.00

Data are means and standard deviation; ANOVA test (P > 0.05)

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