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Analysis of Antagonistic Endophytes and Medicinal Plant Antibacterial

S Madhu, Research Scholar, Department of Botany , J.S University, Shikohabad.

Dr BPS Chauhan, Professor ,Supervisor, Department of Botany, J.S University,Shikohabad.

Abstract-It is likely that medicinal plants have endophytic bacteria due to the presence of bioactive components inside their cells. Within the Chatkal Biosphere Reserve in Uzbekistan, endophytic bacteria were successfully extracted from two different medicinal plants. Both Hypericum perforatum and Ziziphora capitata were identified as these plants. The plant-specific qualities of these bacteria, which are involved in both biocontrol and the stimulation of plant growth, were investigated. This was the preliminary study that was done in preparation for a larger-scale undertaking. Plant extracts of Z. capitata were unable to exhibit any possible antibacterial activity, but extracts of H. perforatum were found to be very efficient against bacterial and fungal infections. Analytical methods such as time-of-flight mass spectrometry (TOF) and matrix-assisted laser desorption ionization (MALDI) were used in order to identify culturable endophytic bacteria that are associated with plants. There are a total of eight distinct genera represented among the cultivable endophytes that have been reported to be associated with H. perforatum. Arthrobacter, Achromobacter, Bacillus, Enterobacter, Erwinia, Pseudomonas, Pantoea, Serratia, and Stenotrophomonas are some of the taxa that fall under this category. Endophytic isolates from Z. capitata have been found to include members of the following taxa, with the exception of Arthrobacter, Serratia, and Stenotrophomonas. In comparison to Z. capitata, H. perforatum was home to a more diverse collection of microorganisms that were capable of producing antagonistic effects. Because the antagonistic isolates were able to inhibit Fusarium oxysporum's ability to produce tomato root rot and encourage plant development in greenhouse environments, they could be a low-cost source of agriculturally-based biological control agents. This is because tomato root rot is caused by a fungus called Fusarium oxysporum. In addition to this, the settings that are found in greenhouses are suitable for the development of plants.

Keywords: Hypericum perforatum, Ziziphora capitata, endophytic bacteria, plant growth traits, antimicrobial activity, antagonism

1. INTRODUCTION

Herbal medicine has been utilized as a therapeutic intervention for a diverse range of ailments for an extensive duration, encompassing but not limited to asthma, gastrointestinal complications, skin disorders, respiratory and urinary tract afflictions, as well as hepatic and cardiovascular maladies (Tian et al., 2014; Van Wyk and Wink, 2004). This particular activity boasts a rich and distinguished lineage. The flora exhibits the ability to endure and flourish in their indigenous

ISSN: 2057-5688

habitat owing to their capacity to generate a diverse array of biologically potent substances (Bajguz, 2007; Cushnie et al., 2014). The compounds in question serve as a means of defense against abiotic stresses, such as those brought about by fluctuations in temperature, water availability, mineral nutrient levels, and insect pests. This has been noted by various scholars, including Simmonds (2003), Treutter (2006), and Vardhini and Anjum (2015). The references cited in the text are Zhao et al. (2011) and Morsy (2014). Studies have indicated that the chemical constituents of pharmacologically active botanicals may exhibit considerable variation contingent upon the plant's taxonomic classification, soil type, and symbiotic relationships with soil microorganisms.

The studies conducted by Qi et al. (2012), Philippot et al. (2013), and Chaparro et al. (2014), as reviewed by Koberl et al. (2013), are relevant to the topic at hand. The potential influence of bioactive secondary metabolites on plant-associated microbial communities and their associated physiological processes cannot be overlooked. The microbiome of plants plays a crucial role in various traits and functions such as growth promotion, nutrient acquisition, induced systemic resistance, and tolerance to abiotic stress factors, as evidenced by the research conducted by Egamberdieva et al. (2010), Malfanova et al. (2011), Sessitsch et al. (2013), and Berg et al. (2014). The microbiome is responsible for a variety of functions and characteristics. Koberl et al. (2014) assert that although numerous studies have been conducted on the phytochemical composition and pharmacological properties of medicinal plants, there is a dearth of knowledge regarding their microbiomes and the physiological interplay between hosts and bacteria.

Beneduzi et al. (2012) and Berg et al. (2014) have reported that the microbiome associated with plants is constituted by diverse microbial communities that inhabit the roots, shoots, and endosphere of the plant. The aforementioned sources (Weller et al., 2002; Berendsen et al., 2012; Philippot et al., 2013) have been cited. Extensive research has been carried out on the rhizospheres of diverse plant species, with the aim of identifying advantageous bacteria that could potentially enhance plant health. The acquisition of knowledge regarding the response of microbial communities to alterations in the physiochemical conditions of the rhizosphere has the potential to provide valuable insights into the microbial ecology of plant-associated bacteria. Koberl et al. (2013) reported that the rhizospheres of Matricaria chamomilla, Calendula officinalis, and Solanum distichum, which are therapeutic herbs, exhibited a noteworthy presence of antagonistic bacteria that impeded the growth of beneficial bacteria. Kumar et al. (2012) reported that the microorganisms residing in the root system of Ajuga bracteosa exhibit diverse plant growth-promoting attributes. The aforementioned effects encompass the generation of siderophores, indole acetic acid, and antioxidant activity. The interdependence between endophytic microbes and host plants is thought to be associated with the phytochemical constituents of plants, either through direct or indirect means, as suggested by Chandra (2012) and Qi et al. (2012). In recent times, there has been a notable surge in research interest towards endophytic bacteria due to their symbiotic association with their host, as highlighted by Hardoim et al. (2015). Despite the initial investigations conducted on endophytes present in plants (Bharti et al., 2012; López-Fuentes et al., 2012; Miller et al., 2012; El-Deeb et al., 2013; Egamberdieva and Teixeira da Silva, 2015), the potential of medicinal plants remains largely unrealized.

2. MATERIALSANDMETHODS

2.1 CollectionofPlantSamples

ISSN: 2057-5688

In the summer of 2013, specimens of the plant species Hypericum perforatum (Hypericaceae) and Ziziphora capitata (Lamiaceae) were procured from the western slopes of the Tien Shan mountain situated within the Chatkal Biosphere Reserve in Uzbekistan (41°08' N, 69°59' E). This biosphere reserve is situated in the Chatkal mountain range of the West Tien-Shan Mountains, specifically in the Tashkent Region. It spans an altitude range of 1,110 to 4,000 meters above sea level. Its uniqueness stems from its significant role in the conservation of ethnobotany and biodiversity. The climate exhibits an elevation-dependent pattern of precipitation, with a gradual increase from lowlands to highlands, peaking at 700-800 mm. The average annual temperature falls within the range of 20-25 °C. The arid climate is prevalent in the plains, whereas the mountainous regions exhibit a comparatively humid climate.

2.2 Preparation of Plant Extracts

The aerial parts of H. perforatum and Z. capitata were subjected to air-drying in a laboratory setting, devoid of direct sunlight, for a duration of approximately seven days at ambient temperature prior to being pulverized into a fine powder using a mortar and pestle. Approximately 10 grams of plant powder were extracted utilizing 50 milliliters of methanol under conditions of reduced illumination and at ambient temperature. The solvent underwent an evaporation process in a rotating vacuum evaporator at a temperature of 40 degrees Celsius, and was subsequently re-dissolved in dimethyl sulfoxide (DMSO). After being subjected to sterilization through centrifugation at 5000 g for a duration of 15 minutes and subsequent filtration via Whatman No. 1 paper, the homogenate was further filtered using sterile filters of 0.22-micron size, which were produced by Millipore, a company based in Bedford, Massachusetts, located in the United States. In vitro analysis was conducted to evaluate the antibacterial efficacy of the filtrates that were stored at a temperature of 4 degrees Celsius.

2.3 Antimicrobial Activity of Plant Extracts

The study involved the evaluation of the efficacy of various extracts against a range of pathogenic microorganisms. The microorganisms tested included Klebsiella oxytoca 6653, K. pneumoniae 40602, K. aerogenes NCTC 8172, Citrobacter freundii 82073, Staphylococcus aureus MRSA 16, Enterococcus faecalis NCTC 775, Providencia rettgeri NCIMB 9570, Pseudomonas aeruginosa NCTC 6749, Escherichia coli NCTC 9001, and Fusarium solani, Fusarium oxysporum, and Alternaria alternata. Each extract was tested individually against these microorganisms. The Department of Microbiology at Manchester Metropolitan University, UK, and the National Culture Type Collection (NCTC), UK, were the sources of the reference strains and clinical isolates. The fungal strains were acquired from the Department of Microbiology and Biotechnology at the National University of Uzbekistan. The plant extracts were dissolved in dimethyl sulfoxide (DMSO), filtered using a sintered glass filter for sterilization, and subsequently stored at a temperature of 4◦C. The study evaluated the antimicrobial efficacy of the extracts through the utilization of the agar well-diffusion technique. The microorganisms were cultured for a period of one night at a temperature of 30◦C in Mueller-Hinton Broth, which was supplemented with 5% horse blood, and subsequently, a suspension of 100 µl containing 106 CFU ml−1 of bacteria was evenly distributed on the Mueller-Hinton agar plates. Cylindrical cavities with a diameter of 6 millimeters were excised and subsequently loaded with 50 microliters of each extract at a concentration of 10 milligrams per milliliter. The controls employed in the study were Ampicillin (Sigma-Aldrich, Steinheim, Germany) at a concentration of 0.5 mg ml−1, nystatin (Sigma-Aldrich, Steinheim, Germany) at a concentration of 1 mg

ISSN: 2057-5688

ml−1, and DMSO. The fungal strains were cultured on potato dextrose agar plates (PDA) obtained from Difco Laboratories in Detroit, MI, USA, and incubated at a temperature of 28◦C for a duration of 5 days. A small quantity of fungal culture was centrally positioned within the Petri dishes. The antimicrobial assay was conducted three times to ensure accuracy and consistency. The Petri dishes were subjected to incubation at optimal temperatures for bacterial strains (37◦C) and fungal strains (30◦C) for a duration of 2 and 4 days, respectively. The evaluation of the antimicrobial efficacy was predicated on the quantification of the zones of inhibition encircling the well on the agar substrate.

2.4 Isolation of Endophytic Bacteria

Twenty-four specimens, comprising three plants from each species of H. perforatum and Z. capitata, were gathered at random from a 100 m2 region within the Chatkal Biosphere Reserve. The collection included roots that were extracted from a depth of 20-30 cm. The intact plants, inclusive of their root systems, were enveloped in plastic bags and expeditiously transported to the laboratory on the same day of collection. Upon arrival, they were promptly preserved at a temperature of 4◦C. Bacterial strains were isolated on the subsequent day to mitigate any potential impact of storage.

The plant root systems were isolated from the shoots, and the soil that was attached to the roots was eliminated. The roots were then meticulously washed under a stream of water, with the aim of reducing any potential damage to the roots. The study employed a sample size of three plants per species to ascertain the quantity of bacterial colonies that were cultured from the root tissue. To perform bacterial isolation, the root tissues were collected from three separate plants and subsequently combined for each of the three replicates. The roots underwent surface sterilization through immersion in a 70% (v/v) ethanol solution, followed by agitation in a 5% (w/v) sodium hypochlorite solution for a duration of 5 minutes. Following that, the roots underwent six rounds of rinsing with sterile distilled water. In order to evaluate the effectiveness of sterilization, the roots that had undergone the sterilization process were subjected to incubation in a TSA medium for a duration of 2 days at a temperature of 28◦C. Upon observation, no instances of infestation were detected.

Aseptic techniques were employed to weigh sterilized roots, which were then macerated in a mortar using phosphate buffered saline (PBS) consisting of 20 mM sodium phosphate, 150 mM NaCl, and pH 7.4, within a laminar air flow cabinet. The samples were suspended in a sterile phosphate-buffered saline solution (PBS) of 9 ml and agitated using a vortex for a duration of 1 minute. The liquid portion of the sample was obtained and subjected to a series of dilutions ranging from 101 to 105 in phosphate-buffered saline (PBS). Subsequently, 100 µl of the diluted samples were evenly distributed on Tryptic Soy Agar (TSA) plates obtained from Difco Laboratories in Detroit, MI, USA, and the process was repeated three times. The specimens were subjected to incubation at a temperature of 28◦C, following which the colony forming units (cfu) g−1 root tissue were evaluated on the third day. A selection of colonies that displayed distinguishable colony morphologies were chosen from the plates and subsequently re-streaked to achieve the isolation and purification of the isolates. The bacterial cultures in their pure form were maintained on plates at a temperature of 4◦C to facilitate subsequent analyses. Furthermore, it should be noted that the bacterial isolates were preserved in Tryptic Soy broth (TSB) (Difco) containing 30% glycerol at a temperature of -80◦C.

2.5 Identification of Endophytic Bacterial Strains

ISSN: 2057-5688

The identification of bacterial isolates was carried out using Matrix-assisted laser desorption/ionization time of flight mass spectrometry, commonly referred to as MALDI-TOF MS, as previously reported by Egamberdieva et al. (2016). The preparation of the sample was conducted in adherence to the ethanol/formic acid extraction protocol recommended by Bruker Daltonics, a company based in Bremen, Germany. The aforementioned methodology can be observed in the study conducted by Egamberdieva et al. (2016). The isolates were cultured on TSA medium (Difco Laboratories, Detroit, Michigan, USA) for a duration of twenty-four hours. Subsequently, they were suspended in three hundred liters of water (LC-MS CHOMASOLVXR; Honeywell) and subjected to vortexing to obtain a uniform solution comprising roughly ten milligrams of cell mass. Following the addition of 900 liters of 99.8% GC purity ethanol obtained from Sigma-Aldrich, the mixture underwent centrifugation. The pellet was meticulously blended with fifty liters of formic acid having a volume-to-weight ratio of seventy percent, and subsequently, it was reconstituted in fifty liters of acetonitrile. Following centrifugation, equivalent volumes of the supernatant, measuring one liter each, were promptly divided into distinct regions on a MALDI target using an aliquoting technique. After the application of 1 liter of the matrix, specifically ciano-4-hydroxycinnamic acid in 50% aqueous acetonitrile with 2.5% trifluoroacetic acid, a drying period was observed for each designated area. In order to acquire mass spectra spanning a mass range of 2-20 kDa, a MALDI-TOF MS spectrometer (MicroflexTMLT, Bruker Daltonics, Bremen, Germany) was employed and operated in linear positive mode throughout the procedure. The calibration of the equipment was performed using a bacterial test standard (BTS) from Bruker Daltonics in Bremen, Germany. The MALDI BiotyperTM software (version 3.0, Bruker Daltonics, Bremen, Germany) was utilized to import the raw spectra and subsequently process and analyze them through standard pattern matching against the reference spectra stored in the database. The scoring value, commonly known as a calculated matching score, served as a measure of the accuracy level of a given classification.

2.6 In vitro Screening for Plant Beneficial Traits

IAA production was quantified by means of a computation utilizing the descriptions provided by Bano and Musarrat (2003). A calibration curve utilizing pure IAA was employed as a reference point to determine the concentration of IAA present in the culture. Pratima et al. (2012) employed a methodology wherein the evaluation of bacterial isolates' ability to degrade cellulose was facilitated by streaking inocula on a Congo-Red agar medium containing cellulose (Sigma-Aldrich, St. Louis, MO, USA). In addition, the substrate utilized for assessing -1,3 glucanase activity in top agar plates (Walsh et al., 1995) was lichenan (Sigma-Aldrich, St. Louis, MO, USA), while protease activity was tested using 5% skimmed milk agar plates (Brown and Foster, 1970). Both of the aforementioned experiments were conducted utilizing top agar plates. The Castric (1975) methodology was employed to assess the bacterial isolates' HCN synthesis.

In vitro experiments were conducted to assess the antagonistic activities of bacterial isolates against various pathogenic fungi and oomycetes, namely Fusarium oxysporumf.sp. radicis-lycopersici (Forl), F. solani, F. culmorum, Gaeumannomycesgraminispv. tritici (Ggt), Alternaria alternata, Botrytis cinerea, and Pythium ultimum. After a period of three days of growth in TSB broth, 50 mL of bacterial cultures were inoculated into a 4 mm diameter hole in PDA plates. The fungal strains were cultured on peptone dextrose agar (PDA) plates at a temperature of 28 degrees Celsius for a duration of five days, in order to facilitate their preparation

ISSN: 2057-5688

for injection. Disks of 5 mm diameter were prepared using newly obtained fungal cultures and placed at a distance of 2 cm from the aperture utilized for the bacterial filtrate. Subsequently, the Petri dishes were sealed with ParafilmXR M and kept in a light-free environment at a temperature of 28°C until the mycelium had colonized the control plates that lacked bacteria. The assessment of antifungal efficacy was conducted by measuring the diameter of the growth-inhibited zone between the studied fungus and bacterium.

3. BIOLOGICAL CONTROL OF TOMATO ROOT ROT

The present study investigated the ability of bacterial isolates to mitigate the incidence of root rot instigated by Fusarium oxysporum f.sp. radicis-lycopersici in tomato plants. The majority of the fungal infections that were tested against these bacteria yielded unfavorable results. Prior to its introduction into the soil, F. oxysporum was cultivated on PDA plates for a duration of five days. The Chapek-Dox media was kept under controlled conditions of 28°C temperature and 110 revolutions per minute air circulation fan speed. The agar was fragmented into small pieces and subsequently blended before being added to the medium. Following a period of three days, the spore solution underwent filtration via sterile glass wool for the purpose of segregating the mycelium from the spores. Prior to the thorough mixing of the inoculum's spore concentration with potting soil to achieve a concentration of roughly 107 spores kg1 soil, a hemocytometer for cell counting was employed to elevate its spore concentration to 107 spores ml1 and attain the intended level. The pink tomato seeds known as Fuji were initially developed in Sakata, Japan. To ensure sterility, the seeds underwent a process involving a five-minute spin in 70% ethanol, followed by a three-minute submersion in a diluted solution of home bleach containing approximately 5% sodium hypochlorite. Subsequently, the seeds underwent multiple washes utilizing sterile, distilled water. Following the process of germination on sterile Petri dishes, the seeds were subsequently introduced to a bacterial suspension with a concentration of 108 colony-forming units per milliliter. The seeds were subjected to agitation for a duration of ten minutes while immersed in the bacterial solution. The experimental design involved four sets of 24 plants for each treatment, all of which were propagated from contaminated seeds and subsequently cultivated in plastic containers. The experimental plants were cultivated under controlled conditions, with a regulated photoperiod of 16 hours of light and 8 hours of darkness, and maintained at a daytime temperature of 28 degrees Celsius and a nighttime temperature of 20 degrees Celsius. Additionally, the relative humidity of the growing environment was maintained at 60 percent. Following a duration of three weeks, the vegetation was extracted, sanitized, and evaluated for indications of foot and root decay. The observed indicators comprised of discoloration and lesion formation on the roots and lower portions of the plant. The absence of any indications of disease was deemed as a characteristic of sound roots.

3.1 PlantGrowthStimulation

A pot experiment was conducted in a plant growth chamber to examine the potential of bacterial isolates to promote plant growth. The experiment was conducted under controlled conditions. As previously stated, the tomato seeds (Solanum lycopersicum, cv. Fuji Pink, Sakata, Japan) underwent a process of surface sterilization. Prior to sowing on plastic Petri plates, the seeds underwent surface sterilization. Subsequently, the seeds were allowed to develop for a duration of four days under dark conditions at a temperature of 25 degrees Celsius. Following an additional overnight incubation in Tryptic Soy Broth (TSB), 1 milliliter of each culture was subjected to centrifugation at 10.000 g for a

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duration of ten minutes to obtain pellets. Following the required modifications, the cell suspensions were adjusted to attain an optical density of 0.1 at 620 nm (0.2 for Bacillus and Arthrobacter), indicating a cell density of approximately 107-108 cells ml1. After being washed with 1 milliliter of phosphate-buffered saline (PBS), the cellular pellets were re-suspended in PBS and adjusted to the desired concentration. Prior to germination, the tomato seeds were aseptically transferred into the bacterial solution using sterile forceps. Subsequently, the amalgamation was stirred in a delicate manner. Following a ten-minute incubation period, the seeds were sown under sterile conditions at a depth of 1.5 centimeters into potting soil that contained 250 mg l1 of nitrogen, 120 mg l1 of phosphorus, and 700 mg l1 of potassium, with a pH of 6.0 (Floragard GmbH, Germany). Non-infected plants were utilized as negative controls in the experiments. The experimental design consisted of three replications, with a total of 18 plants. Each treatment group comprised six plants, and the placement of pots was randomized. Given the conditions mentioned earlier, the growth chamber facilitated the proliferation of plant life.

3.2 Statistical Analyses

A one-way analysis of variance (ANOVA) was conducted on the data using the SPSS-22 statistical program, which was developed by SPSS Inc. in Chicago, Illinois, United States of America. A statistical analysis was conducted to compare means using the least significant difference (LSD) test at a significance level of 0.05.

4. RESULTS

4.1 The Antimicrobial Activity of Plant Extracts

The study evaluated the inhibitory properties of extracts obtained from Z. capitata and H. perforatum against various enteric pathogens, namely A. baumanii 60649, K. oxytoca 6653, K. pneumoniae 40602, K. aerogenes NCTC 8172, C. freundii 82073, S. aureus MRSA 16, E. faecalis NCTC 775, Proteus rettgeri NCIMB 9570, P. aeruginosa NCTC 6749, and E. coli NCTC 9001. The extracts were tested at a concentration of 10 mg ml−1, and the results showed varying degrees of inhibition. The extract of H. perforatum exhibited inhibitory effects against the following strains: A. baumanii 60649, E. coli NCTC 9001, E. faecalis NCTC 775, K. oxytoca 6653, K. pneumoniae 40602, P. aeruginosa NCTC 6749, and S. aureus MRSA 16. Nevertheless, the extract derived from Z. capitata did not demonstrate any discernible antibacterial efficacy against the 10 pathogens that were subjected to testing. The findings suggest that H. perforatum extracts possess promising antifungal properties against F. oxysporum and A. alternata, while Z. capitata extract did not demonstrate any inhibitory effects on the fungal strains examined.

4.2 Enumeration, Isolation, and Identification of Endophytic Bacteria

The total number of endophytic bacterial isolates in the root tissue of Z. capitata was significantly higher (4.5 0.8 103 CFU g−1 of fresh root tissue) than in H. perforatum roots (2.6 0.71 103 CFU g−1 of fresh root tissue). Isolates were chosen randomly from the dilution plates exhibiting different colonial morphology, forms, texture, and color from each plate. A total of 18 bacterial isolates were derived from
H. perforatum and 15 isolates from Z. capitata. Taxonomic investigation by MALDI-TOF MS revealed that the majority of strains were identified with secure genus identification and probable species identification (Table 2). The endophytes from the root of H. perforatum were affiliated with nine genera, whereas 14 isolates were identified at the species level.

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Achromobacter was the predominant genus, which was followed by the genus Pseudomonas. Furthermore, isolates affiliated with the genera Arthrobacter, Bacillus, Erwinia, Pantoea, Serratia, and A total of five bacterial genera were isolated from the root of Z. capitata (Table 1). The most abundant isolates of Z. capitata were also identified as A. piechaudii (M11, M6, M31, M24, M41). Members of the genera Serratia, Stenotrophomonas, and Erwinia were not identified among the endophytes from Z. capitata.

4.3 Biological Control and Plant Growth Promotion

The bacterial isolates that exhibited antagonistic activity against a wide range of fungal pathogens *in vitro* were selected to evaluate their ability to suppress tomato foot and root rot caused by *F. oxysporum* f.sp. *radicis-lycopersici* in a pot experiment. In non-infested soil, the portion of diseased plants was 2%, whereas in the presence of the pathogen, the portion of plants that exhibited disease symptoms increased to 38% (**Figure 2**).

4.4 DISCUSSION

The present investigation involved an analysis of the antimicrobial properties of extracts derived from the plants Hypericum perforatum and Ziziphora capitata. Additionally, the study aimed to characterize the advantageous attributes of the culturable endophytic bacteria associated with these plants. The results indicate a correlation between the parameters, as the Hypericum plant extracts displayed superior antimicrobial activity and a greater prevalence of endophytes with antagonistic activity compared to Ziziphora, which does not possess antimicrobial activity. The study demonstrated that H. perforatum has significant potential as an antimicrobial agent against a diverse array of pathogenic bacteria (including A. baumanii, E. coli, E. faecalis, K. oxytoca, K. pneumoniae, P. aeruginosa, and S. aureus) and fungi (such as F. oxysporum and A. alternata). Conversely, the extract of Z. capitata did not exhibit any inhibitory activity against the microorganisms tested. Maleš et al. (2006) reported a comparable finding for H. perforatum, wherein they discovered that methanol extracts demonstrated potent antibacterial activity against S. aureus, S. epidermidis, E. faecalis, and Bacillus subtilis.

The present investigation revealed a reduced quantity of endophytes in H. perforatum as opposed to Z. capitata, which demonstrated antibacterial properties. The findings presented here align with those of Ahmed et al. (2014), who similarly observed a reduced microbial population in the rhizosphere of M. chamomilla. This plant species is known to exhibit significant antibacterial activity against pathogenic bacteria, as reported by Munir et al. (2014). The results of our study indicate that endophytes' physiological characteristics were differentially impacted by host plants with varying levels of antibacterial activity. The literature has previously documented the production of phytohormones, siderophores, and antifungal compounds by endophytic bacteria in their interactions with host plants. This understanding has been established by several authors, including Berg et al. (2013, 2014), Cho et al. (2015), and Egamberdieva et al. (2015a,b). According to Berg et al. (2014) and Cao et al. (2014), endophytic bacteria have the potential to enhance plant growth through safeguarding plants against soil-borne diseases and various environmental stressors.

INTERNATIONAL JOURNAL OF TECHNO-ENGINEERING
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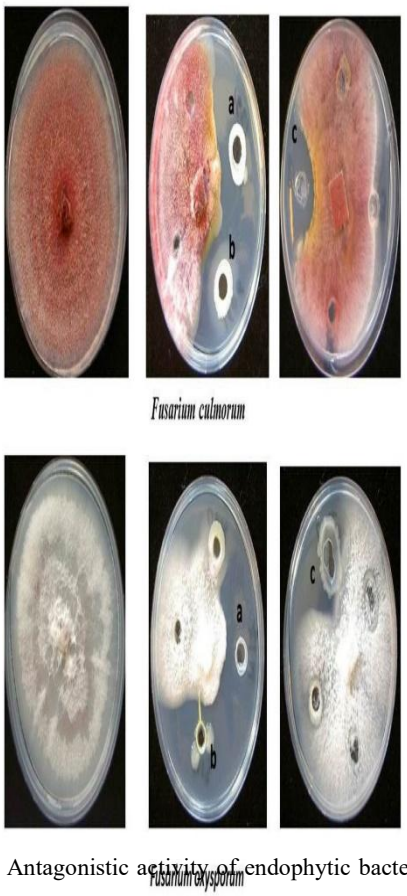

FIGURE 1 | Antagonistic activity of endophytic bacterial isolates. (a) Pseudomonaspseudoalcaligenes S24, (b) Stenotrophomonas sp. S9 isolated

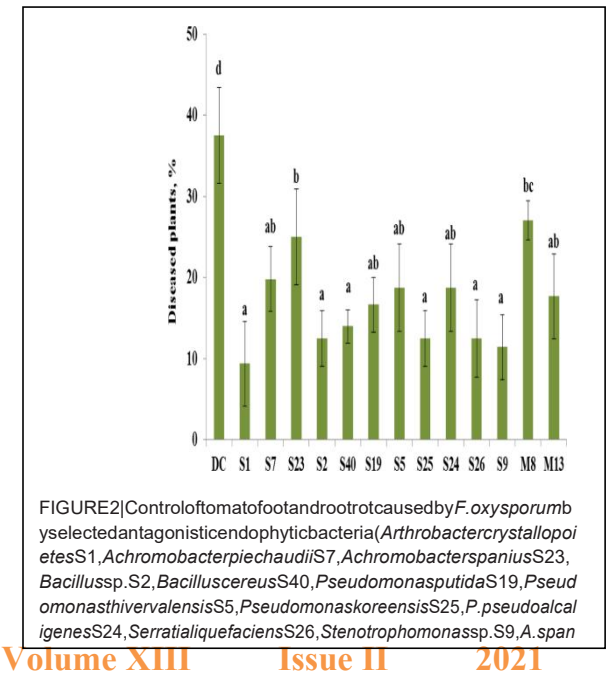

FIGURE2|Controloftomatofootandrootrotcausedby*F.oxysporum*byselectedantagonisticendophyticbacteria(*Arthrobactercrystallopoietes*S1,*Achromobacterpiechaudii*S7,*Achromobacterspanius*S23,*Bacillus*sp.S2,*Bacilluscereus*S40,*Pseudomonasputida*S19,*Pseudomonasthivervalensis*S5,*Pseudomonaskoreensis*S25,*P.pseudoalcaligenes*S24,*Serratialiquefaciens*S26,*Stenotrophomonas*sp.S9,*A.span*

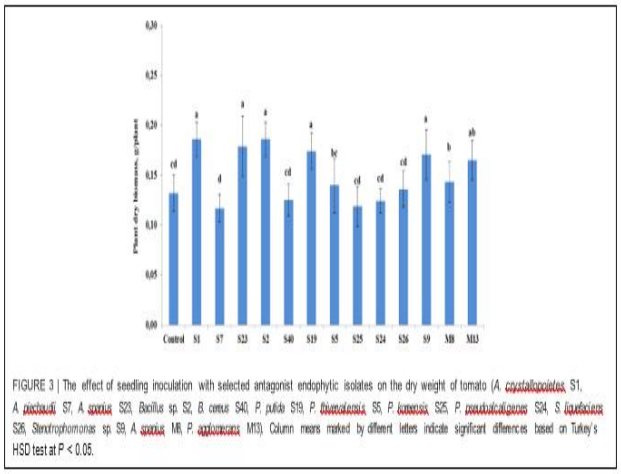

FIGURE 3 | The effect of seedling inoculation with selected antagonist endophytic isolates on the dry weight of tomato (*A. crystallopoietes* S1, *A. piechaudii* S7, *A. spanius* S23, *Bacillus* sp. S2, *B. cereus* S40, *P. putida* S19, *P. thivervalensis* S5, *P. koreensis* S25, *P. pseudoalcaligenes* S24, *S. liquefaciens* S26, *Stenotrophomonas* sp. S9, *A. spanius* M8, *P. asplenicolens* M13). Column means marked by different letters indicate significant differences based on Tukey's HSD test at $P < 0.05$.

The present investigation assessed the potential of endophytic isolates to mitigate F. oxysporum-induced tomato foot and root rot, based on their antagonistic activity against a diverse spectrum of fungal pathogens. The bacterial isolates of A. crystallopoietes S1, Bacillus sp. S2, B. cereus S40, P. koreensis S25, S. liquefaciens S26, and Stenotrophomonas sp. S9, which were selected for the study, demonstrated a significant reduction in disease when compared to the control plants infected with Fusarium, as per the statistical analysis. The aforementioned observations serve as evidence of the ability of endophytes to provide protection to plants against diseases that are transmitted through the soil. The findings indicate that the Verticillium wilt disease of cotton can be controlled through biological means, specifically through the utilization of endophytic bacteria B. subtilis KDRE 01 and B. megaterium KDRE 25. These bacteria were obtained from the medicinal plant Sophora alopecuroides, as reported by Lin et al. (2013). According to Messiha et al. (2007), Stenotrophomonas maltophilia has been identified as an antagonist against Ralstonia solanacearum and has been found to effectively suppress potato brown rot in Egyptian clay soil. In addition, it was observed that five isolates, namely A. crystallopoietes S1, A. spanius S23, Bacillus sp. S2, P. putida S19, and Stenotrophomonas sp. S9, which possess

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antifungal properties, demonstrated a positive impact on the growth of tomato plants. The present discovery aligns with the observations made by Wei et al. (2014) regarding the augmented growth of tomato plants through the utilization of B. subtilis that was extracted from the rhizosphere of Trichosantheskirilowii, a traditional Chinese medicinal herb. Arun et al. (2012) conducted a study wherein they isolated endophytic bacteria from Cassia occidentalis, a widely used weed in traditional medicines. The bacteria were found to produce IAA and were able to promote growth of mung bean in pot experiments.

5. CONCLUSION

The findings obtained from our preliminary investigation of current research offer valuable perspectives on the advantageous characteristics of cultivable endophytic bacteria that are linked to the medicinal plants H. perforatum and Z. capitata, which exhibit varying antimicrobial properties. The findings of our observation indicate that H. perforatum, which possesses antibacterial properties, exhibited a greater capacity to sustain bacteria with antagonistic activity in comparison to Z. capitata. Under greenhouse conditions, it was observed that the antagonistic isolates exhibited the ability to manage tomato root rot, which is caused by F. oxysporum. These isolates could potentially serve as a cost-effective solution for the development of agro-based biological control agents. The aforementioned results suggest that additional investigation is required to thoroughly elucidate the effects of medicinal plant species possessing divergent antimicrobial properties on the endophytic microbial population, as well as to ascertain the bioactive compounds synthesized by the hosts and their endophytes.

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