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Research Article
Screening and Molecular Characterization of Salt Tolerant Bio-control Bacterial Isolates from *Casuarina equisetifolia* Rhizosphere Soil

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Abstract

Background and Objective: *Casuarina equisetifolia* is a fast growing evergreen tree grown by framers under agro-forestry systems in Tamil Nadu state of Southern India. It is encountered by several fungal diseases. The objective of the study was to develop eco-friendly management of the diseases; sixteen different bacterial isolates were isolated from the rhizosphere region of healthy *Casuarina equisetifolia* trees and tested for its salt (Sodium chloride) tolerance and antagonistic potential. **Materials and Methods:** All the isolates were subjected to salt tolerance study, six best isolates were selected and tested its antagonistic efficacy against plant pathogenic fungi viz., *Fusarium oxysporum* (damping off), *Rhizoctonia solani* (damping off) and *Trichosporium vesiculosum* (blister bark) under normal and salt stressed conditions. The presence of bioactive compounds from best isolate was screened using GC-MS analysis. All the data were analyzed using one-way ANOVA and the significant difference among the means were compared by Duncan's Multiple Range Test (DMRT) at p = 0.05 level. **Results:** It was observed that the bacterial isolate KS-21 showed maximum growth retardation of pathogenic fungal mycelium and it was effective in production of diffusible and volatile metabolites against two pathogens, except *Trichosporium vesiculosum* which was least controlled by all the bacterial isolates. There was no marked variation in bio-control activity in sodium chloride amended medium. Based on molecular characterization, the bacterial isolate KS-21 was identified as *Bacillus pumilus*. Ethyl acetate extract fraction of the bacterium exhibited maximum zone of inhibition against *Fusarium oxysporum*. Altogether, 19 bioactive compounds were identified through GC-MS analysis. **Conclusion:** It was concluded that the formulation of bio-fungicide using *Bacillus pumilus* culture or its products will be a good alternative for hazards chemical fungicides.

Key words: Blister bark, *B. pumilus*, *Casuarina equisetifolia*, diffusible metabolite, salinity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity is a major problem causing huge productivity loss in crops and this stressed condition makes crops more susceptibility to disease caused by various pathogens. Agrios[1] reported that more than 10,000 species of fungi can cause diseases in plants. Diseases result in substantial yield losses of agricultural, horticultural and forestry plant species. *Casuarina equisetifolia* is a fast growing multipurpose tree frequently encountered by the blister bark disease caused by *Trichosporium vesiculosum*[2] and damping off disease caused by *Fusarium oxysporum* and *Rhizoctonia solani*[3].

The indiscriminate use of synthetic chemicals against various diseases may cause severe ecological and health issues. Many of the studies in the last decade have focused the discovery of new active principles that generate a lower impact on the environment[4]. Rhizosphere microbes are the best to be used as bio-control agents, since the rhizosphere offers the frontline protection for roots against pathogens. Many microbial antagonists have been reported to have antagonistic actions against different plant fungal pathogens, such as *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens*, *Burkholderia cepacia*, *Pseudomonas fluorescens* and *Trichoderma viride*. They are successfully used for the control of different plant pathogens such as *Alternaria* sp., *Aspergillus*sp., *Botrytis* sp., *Fusarium* sp., *Gaeumannomyces* sp., *Pythium* sp., *Phytophthora* sp., *Pyricularia* sp. and *Rhizoctonia* sp[5].

When compared with biologically active molecules from plants and animals, microorganisms have got importance due to the feasibility of mass culture through fermentation in bioreactors[6]. For bio-control approaches in saline soils, salt tolerance of bio-control agents is an important necessity[7]. While the mode of action for bio-control agents has been well examined, much less is known about the salt tolerant bio-control microorganisms. Hence, in the present study an attempt was made for identification of microbial strains with bio-control potential against important diseases of *C. equisetifolia* grown under salt stressed agroforestry system situated at Karur, Tamil Nadu, South India. The study will reveal the knowledge of active mechanism and secondary metabolites production in salt tolerant microbes.

MATERIALS AND METHODS

Soil sample collection: Soil samples were collected from the rhizosphere of *C. equisetifolia* grown in agro-forestry plantation at Moolimagalam, Karur district, Tamil Nadu, South India lies at N 11°.08′ 00″, E 77°.98′39″. The study was conducted in November, 2013-January, 2014.The soil samples were collected up to 15 cm depth by random sampling method and brought to laboratory in sterile bags and stored in refrigerator at 4°C until further analysis. The methods described by Verma[8] was followed to determine the soil physico-chemical analysis viz., pH, organic carbon, Electrical Conductivity (EC), Nitrogen, Phosphorous, Potassium, Calcium and Magnesium at Soil Testing Laboratory of the Institute of Forest Genetic and Tree breeding, India.

Enumeration of bacterial isolates: Serial dilution and plating techniques as described by Subba Rao[9] with modification were adopted for enumerating bacterial population from soil samples. The Nutrient agar and Trypticase soy agar media with 5% NaCl concentration w as used for enumeration of bacterial isolates. After 24-48 h incubation at 37°C, the colonies appeared with different morphology were selected for further study. The Colony Forming Units (CFU) per gram soil was determined.

Effect of various concentrations of sodium chloride (NaCl) on the growth of isolates: The level of salt tolerance of the bacterial isolates was determined by Nagarajan and Natarajan[10]. The nutrient broth supplemented with various concentrations of NaCl (5, 10,15 and 20%) was inoculated with overnight grown bacterial isolates and incubated for 48-72 h at 37°C. Nutrient broth without extra addition of NaCl was maintained as control. Optical density at 600 nm was measured using UV-Vis spectrophotometer (HITACHI, U-2000 series).

***In vitro* evaluation of antagonistic potential of bacteria using dual culture method:** Pathogen cultures of *F. oxysporum*, *R. solani* and *T. vesiculosum* isolated from diseased samples of *C. equisetifolia* were grown on Potato Dextrose Agar (PDA) at 28°C for 96-120 h, pure cultured and stored at 4°C for further study.

The selected bacterial isolates were assessed for their potential bio-control activity using dual culture technique[11]. An agar disc of 4 mm was cut from an actively grown 96 h pathogen culture and placed on the surface of fresh PDA medium with different concentrations of NaCl (0, 5, 10, 15 and 20%) at the centre of the petri plates. A loopfull of actively growing bacterial isolate was streaked opposite to the fungal disc, approximately 3 cm from the centre. The petri plates inoculated with the plant pathogen and without bacterial

isolates were kept as control. All the petri plates were incubated at room temperature for 7 days. Degree of antagonism was determined by measuring the radial growth of the plant pathogen with bacterial culture and control. The percentage of mycelial growth inhibition was calculated using the following formula described by Rahman *et al.*[12].

$$\text{Inhibition (\%)} = \frac{C - T}{T} \times 100 \quad (1)$$

Where:

C = Mycelial growth of the plant pathogenic fungus in control plate without bacterial isolate

T = Mycelial growth of the plant pathogenic fungus with bacterial isolate

Effect of diffusible and volatile metabolites on the growth of fungal pathogen: To evaluate the production of diffusible antibiotic compounds from bacterial isolate, 1 mL of overnight grown bacterial culture was transferred into 50 mL nutrient broth and shaked at 37°C for 6 days in shaker orbital incubator. The bacterial culture was centrifuged and supernatant was mixed with melted PDA. A 5 mm disk of a pure culture of fungal pathogen was inoculated at the centre of the petri plate and incubated at 28°C for 5 days[13].

To evaluate the production of volatile compounds, the antagonistic bacterial suspensions were streaked on petri plates containing nutrient agar and 2% glucose. After incubation at 28°C for 24 h, other petri plates containing PDA were inoculated in the centre with a 5 mm disc of a 4 day old fungal pathogenic culture. The two petri plates with no tops were placed face to face, sealed together and incubated at 28°C for 5 days[13]. The experiments for each selected antagonistic isolates were performed against all fungal pathogens with at least three replications. The percent of inhibition was determined by measuring the diameter of fungal mycelium and compared with the growth of control (without bacterial isolate).

Molecular characterization of the best isolate: The 16S rRNA sequences of selected salt tolerant antagonistic bacterial isolate was performed by following Edwards *et al.*[14], Edgar[15] and Cho *et al.*[16] methods. The obtained sequence was blast using NCBI blast similarity search tool and the sequences were deposited in Gene Bank.

Preparation of inoculum and extraction of cured metabolites: As per the procedure followed by Battu and Reddy[17], the inoculum preparation and metabolite extraction from selected bacterial isolate was carried out. The selected bacterial isolate was grown in 100 mL nutrient broth under shaking condition (120 rpm) at 28°C for 120 h. The culture was centrifuged at 10,000 rpm for 15 min to get the cell free filtrate. These culture filtrates were used to study the efficacy against fungal pathogens. Crude metabolites were extracted from the effective growth medium by partitioning with organic solvents viz., chloroform, petroleum ether, ethyl acetate, hexane, acetone and methanol[18].

Partial purification of bioactive compound using Thin Layer Chromatography: Silica gel 60 F_{25-4} alumina backed plates (Merck) (10×10 cm) was used for separation and identification of inhibitory fractions from solvent extracted bacterial broth culture[19]. The solvent system used to separate the compounds was Chloroform: Methanol in 9:1 ratio. The aliquots (10 µL) of each extract were spotted and the solvent front was allowed to run for 1/4th of the Thin Layer Chromatography (TLC) plate. The running lane was then air dried thoroughly and elution of compound detected at 254 nm. The RF value was calculated for all bands and obtained bands were scraped into different micro-centrifuge tubes and extracted with appropriate solvent used for extraction. Centrifugation was carried out to remove the silica residue, the supernatant was collected and stored for further use.

Antifungal property of TLC fraction: The individual fractions were tested for antifungal activity by well diffusion method described by Valgas *et al.*[20]. The PDA plates were swabbed with fungal pathogen (*F. oxysporum*) and 100 µL extract was filled in the well, incubated for 28°C for 48 h. The zone of clearances was measured and the fraction showing maximum zone of inhibition stored for further study.

GC-MS analysis of effective TLC fraction: Most effective TLC fraction was analyzed using GC-MS to predict the presence of inhibitory compounds in TLC purified fraction obtained from the bacterial isolate KS-21. The GC-MS (Thermo Trace GC Ultra VER: 5.0) equipped with a DB35-MS fused silica capillary column and GC interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70 eV was used. Helium gas was used as carrier at a constant flow rate of 1 mL min^{-1} and 2 µL sample injected; injector temperature was 250°C and ion source temperature of 200°C maintained. The oven temperature was programmed from 70-200°C at the rate of 6°C min^{-1}, held isothermal for 1 min

and finally raised to 260°C at 6°C min^{-1}. Interface temperature was kept at 250°C. Total GC run time was 40.51 min. The relative percentage of the extract constituents was expressed as percentage with peak area normalization. Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Statistical analysis: All data were analysed using one way analysis of variance (ANOVA) and the significant difference among the means were compared by Duncan's Multiple Range Test (DMRT) a t $p = 0.05$ level using SPSS PC+ Student ware (Version, 1990), statistical software PSS Inc. SAS[21].

RESULTS

Soil characterization: The soil texture of the study area was found to be sandy loam and pH was neutral (7.3) range. The Electrical Conductivity (EC) of the soil was found to be 8.0 dS m^{-1}, it indicated the salinity of the soil. Organic carbon was very low in the range of 0.95%. It is the major factor indicated the health of the soil. Available nitrogen was found to be low (53.6 kg ha^{-1}), phosphorus and potassium were in normal range 24.9 and 995.6 kg ha^{-1}, respectively. The presence of calcium and magnesium was in the range of 66.8-100 g and 14.0 mEq/100 g.

Enumeration of bacterial population: The Colony Forming Units (CFU) per gram of soil in nutrient agar was 50.1×10^6 and in Trypticase soy agar it was 40.5×10^6. A total of 10 colonies with different morphology from nutrient agar and 6 colonies from Trypticase soy agar plates were selected, pure cultured and stored for further analysis.

Screening for salt tolerance: The salt tolerant study with various concentrations of NaCl showed different levels of tolerances in bacterial isolates. All the bacterial isolates showed maximum growth in nutrient agar and difference was observed in salt incorporated medium. Among sixteen isolates, KS-21, KS-23, KS-24, KS-25, KS-28 and KS-33 exhibited significant salt tolerances up to 20%, the different salt tolerance capacity of all the isolates was represented in Fig. 1.

***In vitro* dual culture assay:** There was only slight difference in antagonistic property of bacterial isolates under normal and in saline condition. Among six isolates tested, the bacterial isolate KS-21 exhibited maximum percent of inhibition against all the pathogens. Range of inhibition was found to be high against *F. Oxysporum* followed by *R. solani*. All the isolates showed minimum inhibition against *T. vesiculosum* in the

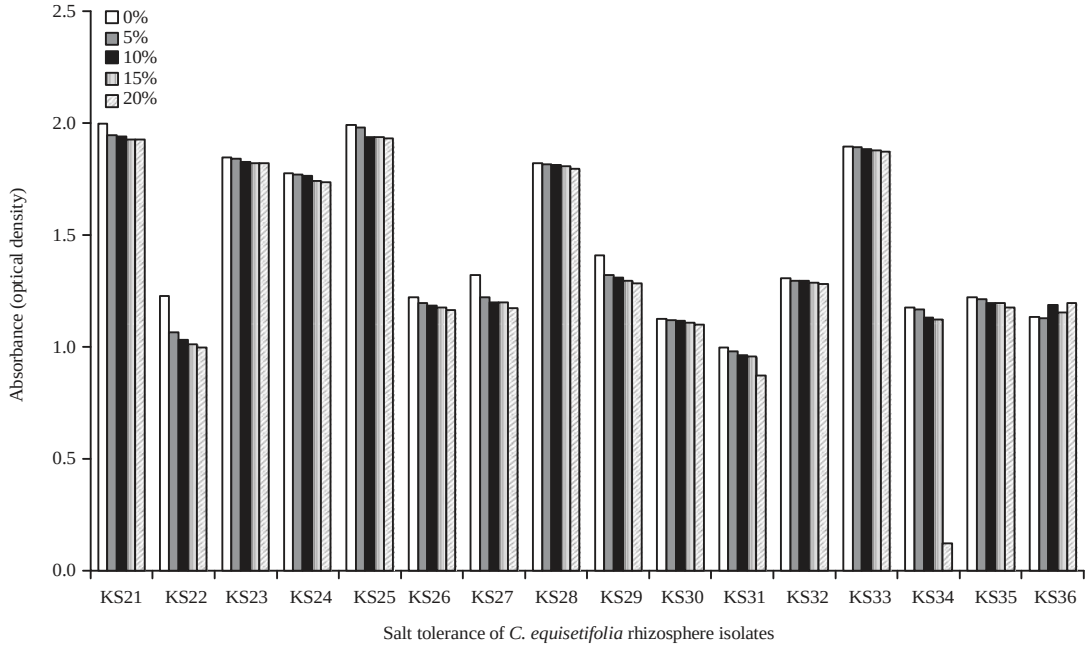

Fig. 1: Effect of different concentration of NaCl on growth performances of *C. equisetifolia* rhizosphere isolates

range of (6-25%) The isolate KS-28 showed no inhibition against *T. vesiculosum* in salt amended medium. The presences of different concentrations of NaCl showed only minor variation in antagonistic property (Fig. 2-4). It proved the salinity tolerance of selected bacterial isolates.

Diffusible and volatile metabolites production: Diffusible metabolites from all the bacterial isolates showed significantly different (p = 0.05) antifungal activity against three fungal pathogens screened. There was no variation in antagonism of dual culture assay, diffusible and volatile metabolites productions were carried out without amendment of NaCl and it was observed that the level of mycelial growth inhibition was found to be high when compared with dual culture method. The filtered broth culture of KS-21 isolate showed significantly higher (p = 0.05) mycelium growth reduction against all fungal pathogens, followed by other bacterial isolates of KS-23, KS-24 and KS2-5. But minimum inhibitory

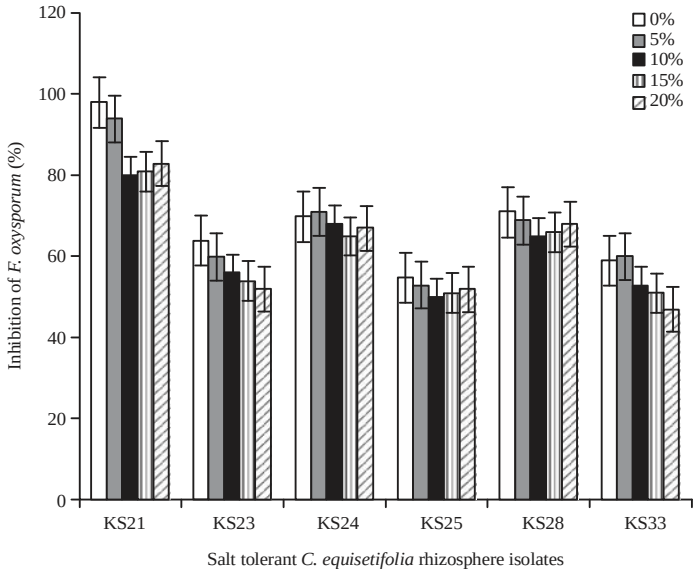

Fig. 2: Percent of inhibition of *F. oxysporum* against *C. equisetifolia* rhizosphere isolates under salt stress

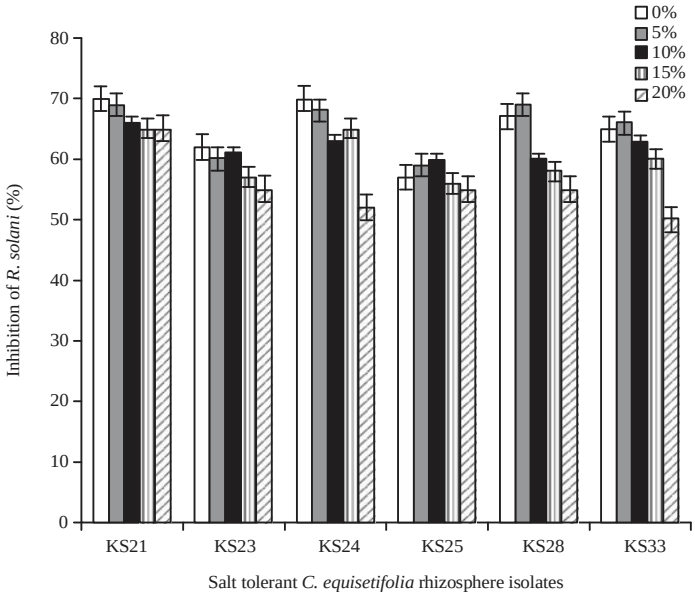

Fig. 3: Percent of inhibition of *R. solani* against *C. equisetifolia* rhizosphere isolates under salt stress
The mean of five replicates and bars indicates standard error

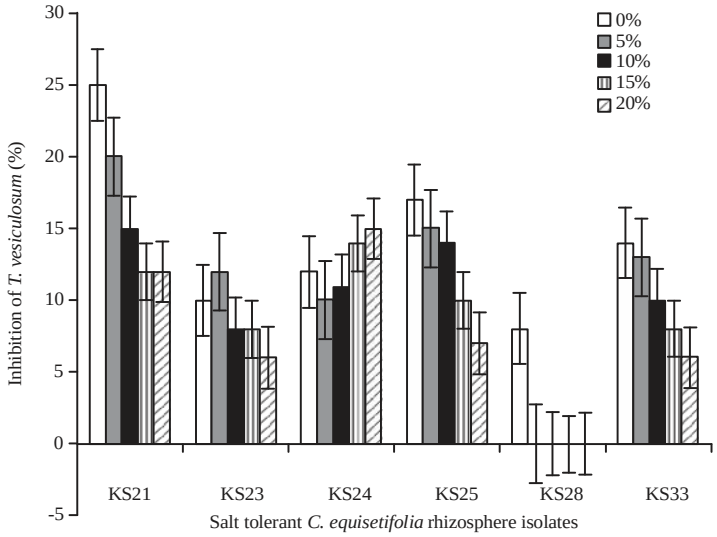

Fig. 4: Percent of inhibition of *T. vesiculosum* against *C. equisetifolia* rhizosphere isolates under salt stress
The mean of five replicates and bars indicates standard error

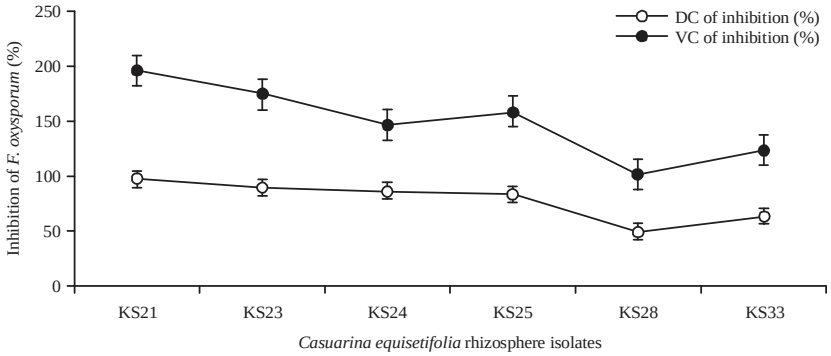

Fig. 5: Effect of Diffusible Compounds (DC) and Volatile Compounds (VC) on the growth inhibition of *F. oxysporum*
The mean of five replicates and bars indicates standard error

effect was found against *T. vesiculosum*. The isolates KS-28 and KS-33 did not have any impact on *T. vesiculosum* but growth of remaining two fungal pathogens was found to be controlled effectively. All the bacterial isolates exhibited maximum retardation of mycelium growth of *F. oxysporum* followed by *R. solani*. The results of the study revealed that the diffusible culture filtrates have an active compound which effectively inhibits the mycelia growth of fungal pathogens.

The volatile compound produced by six bacterial isolates also showed different antifungal activity. The bacterial isolate KS-21 showed effective inhibition of all the tested fungal pathogens, except *T. vesiculosum* where the growth was found to decrease when it was treated with volatile metabolites, the bacterial isolates KS-28 and KS-33 have no effect on *T. vesiculosum* and details are given in Fig. 5, 6 and 7.

Molecular characterization of salt tolerant antagonistic isolate (KS-21): Based on BLAST similarity search tool, the sequence shows closest relativity with *Bacillus pumilus*. The nucleotide sequence determined in this study has been deposited in GeneBank under accession number KX023322.

Assessment of antifungal activity: Ethyl acetate extract of *B. pumilus* showed band with R_F value 0.82 obtained from TLC. Activity of band exhibited maximum zone of inhibition (31 mm) against *F. oxysporum*, followed by Hexane (22 mm) and Methanol extract TLC band (15 mm), no inhibition was observed in petroleum ether TLC band.

Identification of bioactive compounds: Ethyl acetate extract of *B. pumilus* revealed 19 biologically active compounds. The GC-MS peak area of compounds were represented in

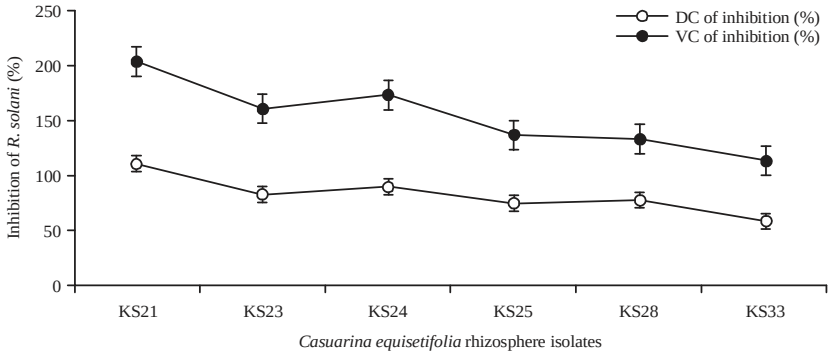

Fig. 6: Effect of Diffusible Compounds (DC) and Volatile Compounds (VC) on the growth inhibition of *R. solani*
The mean of five replicates and bars indicates standard error

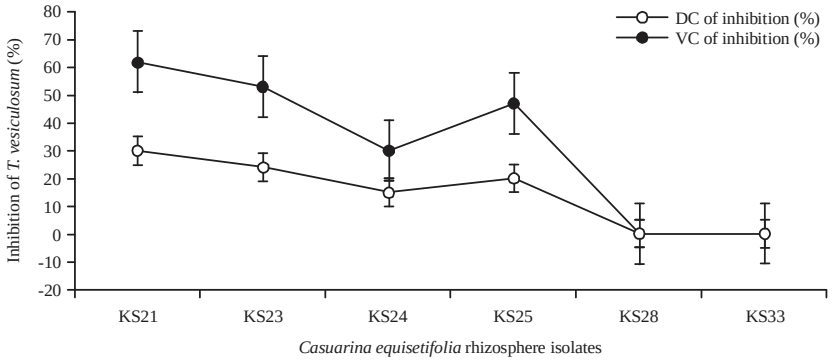

Fig. 7: Effect of Diffusible Compounds (DC) and Volatile Compounds (VC) on the growth inhibition of *T. vesiculosum*
The mean of five replicates and bars indicates standard error

Table 1: Bioactive compounds of isolate KS-21 identified by using GC-MS

Sl. No.	Compounds name	Molecular formula	Molecular weight (g mol^{-1})	Probability	Area (%)	Retention time
1	Cis-3-chloroall alcohol	C_3H_5ClO	92	38.48	32.93	3.14
2	3,4-Dihydro-2H-1,5-(3-t-butyl)benzodiazepine	$C_{13}H_{18}O_2$	206	18.83	2.34	3.98
3	Syn-2-prop-1-3-diol	$C_9H_{20}O_2$	160	42.01	0.67	4.98
4	3-Octadecene	$C_{18}H_{36}$	252	4.21	1.58	7.69
5	Hexanal diethyl acetal	$C_{12}H_{26}O_2$	202	5.63	1.48	8.87
6	3-Methyl-5-lanosta-4,8-dien-3-one	$C_{30}H_{50}O$	426	38.60	0.85	9.75
7	1-Tridecanol	$C_{13}H_{28}O$	200	5.65	0.51	13.45
8	1,3-Bis(4-chlorophenyl)-1,4-dihydrobenzo[f]quinazoline	$C_{26}H_{20}Cl$	430	89.72	0.76	14.43
9	3-Phenl-5-t-butyl pridazine	$C_{14}H_{16}N_2$	212	14.85	1.09	19.20
10	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS)	$C_{16}H_{22}O_4$	278	21.82	3.34	22.89
11	7,9, Di tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	89.82	0.51	23.93
12	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154	61.16	2.40	24.98
13	9-Elcosene	$C_{20}H_{40}$	280	3.39	1.83	25.56
14	1-Nonadecene	$C_{21}H_{42}$	294	6.19	2.10	28.96
15	7-Bromo-2-(4-Fluoro-Benzylidene)-Thia zolo[2,3:2,3]Imidazo [4,5-b]Pyridin-3-one	$C_{15}H_7BrFN_3OS$	375	5.07	4.23	31.38
16	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	$C_{24}H_{38}O_4$	390	19.15	7.95	33.24
17	13,14-Dichloro-behenamide	$C_{22}H_{43}Cl_2NO$	407	27.35	1.75	34.28
18	Melithiazol D	$C_{20}H_{28}N_2O_4S_2$	424	5.35	0.53	36.56
19	9-Octadecenamide, (Z)-(CAS)	$C_{18}H_{35}NO$	281	33.80	20.61	39.19

Fig. 8 and its molecular formula, molecular weight, retention time are given in Table 1. The compounds identified and occurred in higher probability are 1,3-Bis (4-chlorophenyl)-1,4-dihydrobenzo[f]quinazoline (89.72), Syn-2-prop-1-3-diol

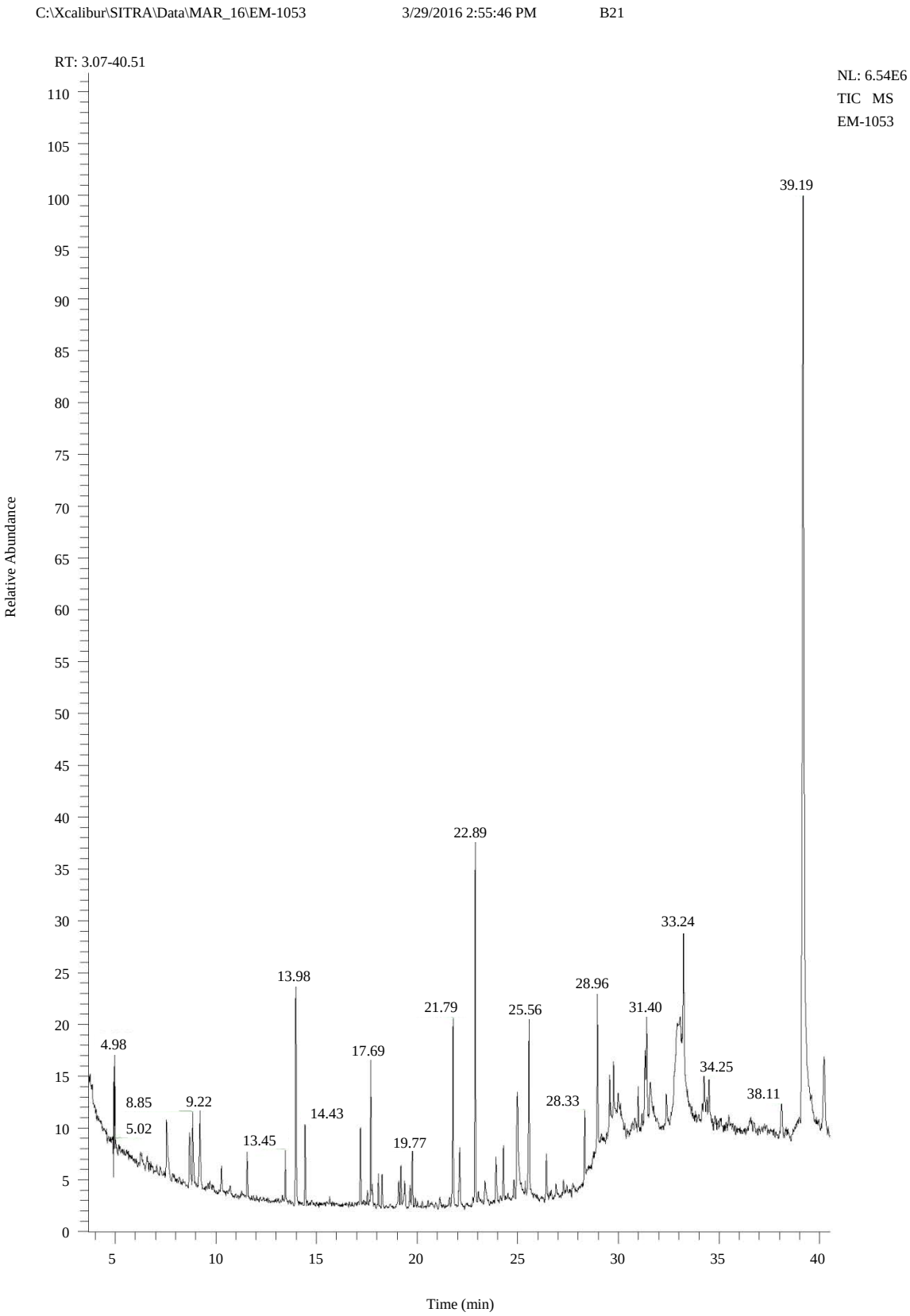

Fig. 8: GC-MS analysis of active compounds from KS-21 ethyl acetate extract
The mean of five replicates and bars indicates standard error

(42.01), 3-Methyl-5-lanosta-4,8-dien-3-one (38.60), 9-Octadecenamide (33.80%), Cis-3-chloroall alcohol (32.93%).

DISCUSSION

The higher EC and deficient nitrogen level observed in soil used for bacterial isolation indicated higher salinity level. Mousivand *et al.*[22] reported that the different soil physico-chemical conditions can influence the bacterial cell number. Similar approach was carried out by Upadhyay *et al.*[23] and they isolated 24 saline tolerant rhizobacterial isolates from saline infested zone of wheat rhizosphere. They found that the isolates could withstand upto 8% NaCl concentration and screened its PGP activity. But in the present study, 16 bacterial isolates obtained from the rhizosphere of *C. equsetifolia* showed growth tolerance upto 12% NaCl.

In this study, isolate KS-21 showed effective antagonistic activity against all the tested plant pathogens when compared with other bacterial isolates. Williams and Asher[24] reported that different antagonistic activities of bacterial isolates possess various mode of actions and the antifungal metabolites produced by the isolates may differ and the isolates are taxonomically different from each other. Edwards *et al.*[25] found that the genera belong to *Bacillus* and *Pseudomonas* was effective against fungal plant diseases. In the present study, they have suggested that production of antifungal compounds by the bacteria may be the cause for the constraint of fungal hyphal growth even there was no direct contact between bacterial colonies and pathogenic fungal mycelium, so that the fungal growth restriction was triggered by diffusion of substances into the agar medium. These results are in agreement with the findings made in the present study. Recently, Shrivastava and Kumar[26] reported that microorganisms with stress tolerance capacity and plant growth promotion attributes are very helpful in growth and development of plants under stressed environments.

Mousivand *et al.*[22] observed that bacteria produce different antifungal metabolites with numerous antagonistic mechanisms and confirmed that the genetic diversity of different patho-types affect the bacterial antagonistic features. Various studies demonstrated that bacterial volatiles provide resistance to salt and droughtstress[27]. The present study was in conformity with the above findings.

In the present study, the potent salinity tolerant bacterial isolates with antagonistic property was sequenced through 16S rRNA and identified with online BLAST in NCBI and it was identified as *Bacillus pumilus*. A similar finding was made by Upadhyay *et al.*[23] and they have reported the occurrences of *Bacillus* and *Bacillus* derived genera were found to be leading in root zone of wheat in saline soil. Spore-forming bacteria, typically *Bacillus* species are one of the major groups of soil bacteria. The production of a multilayered cell wall structure, formation of stress resistant endospores and secretion of peptide antibiotics, peptide signal molecules and extracellular enzymes are the common physiological properties to survive in stressed condition[28]. Quantitative and qualitative variations in these traits allow for these bacteria to inhabit varied niches in agro ecosystems. *Bacillus pumilus* isolated in this study has salt tolerant capacity as well as antagonistic ability against different plant pathogens.

Investigation on microbial metabolites is gaining greater momentum in the agrochemical industry as a source for the development of new pesticide and fungicidal products. Several such products have been developed and used as bactericide, fungicide and insecticide in agriculture. A similar study was conducted by De Melo *et al.*[29] with different fungal pathogens and in their work they have used ethyl acetate and dichlromethene extract of *B. pumilus* for antifungal activity which showed maximum inhibition against *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii*.

In the present study, 19 active compounds were identified from *B. pumilus* through GC-MS analysis. Among them, Cis- 3-chloroall alcohol was one of the compounds with high probabilities (38.48). Belser and Castro[30] reported the biocidal property of this compound. Pyrrolo [1,2-a] pyrazine-1,4-dione (61.16) have anticancer activity. This compound was also identified in ethyl acetate extract of *Micrococcus luteus* by Sheela and Uthayakumari[31]. Similar compound was identified from *B. pumilus* isolated from mangrove sediment and its inhibition in bio-film formation was noticed by Mithun and Rao[32]. In the present study 9-Octadecenamide (33.80) was isolated from the sponge associated actinomycetes, Nocardio psisdassonvillei MAD08 which had the broad range of antimicrobial activity including anticandid activity reported by Selvin *et al.*[33].

In the current scenario the exploration for different microbes and their new bioactive compounds has gained momentum and has great potential in agricultural field for crop protection and enhancing yields. Thus, the *B. pumilus* strain identified in this study could be used in eco-friendly disease management of *C. equsetifolia* raised in salt stressed condition.

CONCLUSION

A good salt tolerant bio-control agent should be able to thrive and persist in salt affected environments. In the

present study, the salt tolerant bio-control bacterial isolate from *C. equisetifolia* rhizosphere was found to be effective in controlling major fungal pathogens. However this *in-vitro* result suggested the use of *B. pumilus* and its secondary metabolites as best eco-friendly alternative against hazards chemical amendments for the control of fungal diseases in salt affected agroforestry systems.

SIGNIFICANCE STATEMENTS

This study discovers the secondary metabolites production of *Bacillus pumilus* isolated from *C. equisetifoila* rhizosphere soil that can be beneficial for formulation of bio-fungicides against major fungal diseases. This study will help the researcher to uncover the critical areas of salt tolerant antagonistic rhizosphere microbes isolated from saline agroforestry system that many researchers were not able to explore. Thus a new theory on use of this bacterial culture or its secondary metabolites as bio-fungicide can benefit farmers to grow tree plantations in salt affected areas.

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