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Fungal Antagonism and Plant Growth Promoting Efficacy of *Aspergillus oryzae* AVNF4 Isolated From the Rhizome of *Curcuma longa* on *Lycopersicum esculantumL*. (Tomato)

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Abstract

The present study aimed to develop liquid bioformulation of *Aspergillus oryzae* AVNF4, *a Curcuma longa* rhizome endophytic fungus with antagonistic activity against *Fusarium oxysporum* causing tomato wilt and plant growth promotion of *Lycopersicum esculantum*. Fungal antagonism, Indole 3-acetic acid production, ammonia and inorganic phosphate solubilisation and GC-MS analysis of antifungal metabolites of *A. oryzae* AVNF4 were analysed. Liquid bio formulation of *A. oryzae* AVNF4 (LBF) with enhanced production of IAA, ammonia and inorganic phosphate solubilisation has developed by optimising the culture medium. Seed bacterisation of tomato seeds with LBF for 48h followed by foliar application from the second week onwards showed a significant increase in germination percentage (Gp) and germination index (GI) along with biomass, plant height, shoot length, root length, root/shoot ratio, number of leaves, and soluble protein and reduced sugars in LBF treated plants compared to untreated control. Presence of 1,3-Dioxolane, 2-(1-propenyl)-, L-Prolinamide, 5-oxo-L-prolyl-L-phenylalanyl-4-hydroxy-, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, dl-Mevalonic acid lactone, Hydro cinnamic acid, Oleic acid, 9-Octadecenoic acid (Z)-, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-, 1-Allylazetidine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-Pyrrolidino-2-pyrrolidone, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methyl propyl)-, 9-Octadecenoic acid (Z)-, hexyl ester, Dehydromevalonic lactone, Acetaldehyde, (3,3-dimethyl cyclohexylidene)-, (Z)- in ethyl acetate extract of AVNF4 reported to be the pivotal compounds responsible for fungal antibiosis and to promote growth in tomato seedlings. The field performance of LBF of AVNF4 indicated that *A. oryzae* AVNF4 is a promising plant growth-promoting endophyte with fungal antagonism against *Fusarium wilt*.

Article History

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Keywords

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Seedling Vigour.

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Introduction

The second most important cultivated vegetable crop globally is tomato, around 115.95 million tons per year, as it is rich with vitamins A and C.[1-4] Tomatowilt is caused by *Fusarium oxysporum*. *Lycopersici* is one of the most destructive and economically damaging diseases, and it is responsible for reducing the quality and quantity of tomato production. *F. oxysporum*. *Sp. lycopersiciis* is a common soil-borne fungus causing severe injury through all phases of tomato growth as it is found in infested agricultural soils, leading to economic losses with characteristic symptoms including wilting, chlorosis, stunted seedlings.[3, 5-11] The usage of chemicals is neither economical nor environmentally friendly, and excessive misuse of fungicides has led to the development of resistance against fungicides.[12,13] *Fusarium* wilt damage has stimulated researchers for biological control using fungi or bacteria, which are antagonistic non-pathogenic microorganisms that have the potency to reduce harmful effects of wilt diseases, which is an alternative to chemical control.[14-17]

Endophytes are powerful biostimulants in inhibiting pathogens, enhancing environmental stress tolerance through an antioxidant system and promoting plant growth, nutrient availability and yield of the crop through the production of indole acetic acid, siderophore, volatile compounds, inorganic phosphate solubilization.[17,18-20] Plant growth-promoting fungi effectively control fungal phytopathogens, including *F. oxysporum*, by producing antioxidants and phytohormones and minimising mycotoxin production.[18,21-23]

Utilising microbes to enhance plant growth and combat plant pathogens is an increasingly recognised concept, offering new insights into sustainable agriculture and reducing the reliance on chemical fertilisers and pesticides. In the past few years, the development of microbial bioinoculants to promote plant growth and eliminate pathogens has emerged as an alternative. Bioformulation research focuses on microorganisms with multiple potentials to inhibit plant pathogens, encourage plant growth, and help to enhance the fertility of soil as an environment-friendly approach.

Hence, this research aims to characterise an endophytic fungus, *Aspergillus oryzae* AVNF4, out of four fungi isolated from the rhizome of *Curcuma longa*, its potential in fungal antagonism against *Fusarium* wilt, and the development of liquid bioformulation to promote the growth of tomato seedlings in pot experiments under greenhouse conditions.

Materials and Methods

Isolation of Curcuma Endophytic Fungi

The endophytic fungus was isolated from rhizomes of *Curcuma longa* collected from the local agricultural field of Nuthakki(16.4158° N, 80.6507° E), village Guntur, A.P. Rhizome was washed to remove dust, debris and soil or clay particles adhered to it with tap water and distilled water. 1g of sterilised rhizome was smashed with 1 ml of 0.1% NaCl, and then 9 ml of 0.1% NaCl was added. Fungus was isolated on Sabroud's medium by serial dilution, and the isolate was confirmed as the endophyte described earlier 24. The last rinsed aliquot (100 µl) was added to Sabroud's Petri plate for control.

Identification of Endophytic Fungal Strain

The physiological characterisation was done by a qualitative screening of hydrolytic enzymes, i.e., amylase, cellulase, protease, lipase, urease, gelatinase, and catalase25followed by18srRNA gene amplification using a universal primer NS1(5' GTA GTC ATA TGC TTG TCT C 3'), and NS24 (5' TCC GCA GGT TCA CCT ACG GA) 3', and NS24 5' (TCC GCA GGT TCA CCT ACG GA 3'). A commercial company, Macrogen, sequenced the amplified genes. Seoul, South Korea. Based on 100% sequence similarity, a phylogenetic tree was constructed using the neighbour-joining method. After identification, AVNF4 was deposited at NCBI, India.

Antifungal Activity

The antifungal activity of the endophytic fungal isolate was screened against *F. oxysporum* using a dual culture assay. *F. oxysporum* mycelium of 1 mm length was spot inoculated at the centre of Sabroud's dextrose agar (SDA) plate, and the endophytic fungal isolate was streaked on either side of the *F. oxysporum* spot at a distance of 3 cm and incubated at 35±2 °C for 7 days. SDA plate spot inoculated with the *F. oxysporum* alone was served as control26. The per cent inhibition of the growth of pathogen was determined by using the formula:

Inhibition of mycelial growth (%) = [X/(X-Y)] X100

Where X and Y are radial growth of mycelia of the pathogen in the absence of an antagonist and the presence of an Antagonist

Plant Growth-Promoting Traits

Plant growth-promoting traits (PGP) such as Indole-3-acetic acid (IAA), Ammonia (NH4+) production and Inorganic PO4-solubilisation (IPS) were screened both qualitatively and quantitatively.

IAA production was qualitatively determined by the Salkowski reagent method using SDA and L-Tryptophan (1 µg ml-1)[27] after 7 days of incubation. The culture supernatant was separated by centrifugation at 10,000 rpm for 15-20 min. The supernatant(1ml)was mixed with one drop of orthophosphoric acid and 2 ml of Salkowski reagent (150 ml H_2SO_4, 7.5 ml FeCl3.6H2O 0.5 M in 250 ml distilled H_2O)and incubated for 30 min. The appearance of pink determined IAA and measured the quantity spectroscopically at 530nm using the IAA standard curve.[28]

IPS was qualitatively determined using a Nutrient agar medium supplemented with tricalcium phosphate. A loop full of culture was placed on the centre of agar plates and incubated at 30±10°C for 5 days. Inorganic phosphate solubilisation was confirmed as a clear zone around the fungal colony[29] and was determined quantitatively using Bartons reagent[30]

NH_4^+ production was tested in peptone agar media using Nessler's reagent (0.5 ml) on the plate. Ammonia production was confirmed by the development of a brown to yellow colour, and quantitative production was determined using 10 ml of peptone water incubated for seven days at 35 ± 2°C by the Nesslerization method.[31]

Optimization Studies

Analyzed growth optimization at different pH ranging 5,6,7,8 and different temperatures (25°C, 30°C, 35°C, 40°C, 45°C), 1% carbon sources (starch, sucrose, fructose, mannitol, dextrose, lactose, glucose, glycerol and maltose) and 0.5% of different nitrogen sources (peptone, yeast extract, beef extract, urea, ammonium chloride, ammonium sulphate, potassium nitrate, and sodium nitrate) in culture broth. Growth was measured in terms of biomass after seven days of incubation.[24]

Development of Liquid Bioformulation

A liquid bioformulation of *A. oryzae* AVNF4 was developed in the optimised SD media on a shaker at 180 rpm and 35 °C for 7 days.[32]

Greenhouse Studies

Preparation of Bacterial Cell Suspension

Royal variety seeds of tomato (Lycopersicon esculentum L.)were selected for greenhouse studies. Seeds were surface sterilised with 5% sodium hypochlorite and 70% ethanol, followed by distilled water rinse for 5times.[33] Seed bacterisation was done by soaking 50 ml of freshly prepared AVNF4 bioformulation for 48 h. The SD broth without fungal inoculation was used as a control treatment. The seeds were sowed in plastic pot trays containing 98 cavities filled with well-sterilised coco peat. Seedling vigour index or germination percentage (Gp), speed of seed germination (S), germination rate (GR), and germination index (GI) were analysed after the first week of germination.

Pot Experiment

The study was carried out in an open greenhouse condition. Weekly foliar application of the liquid bioformulation was administrated from the second week. This pot experiment was done using the randomised method, ensuring seven replicates of each treatment. The plants were uprooted from AVNF4 liquid bioformulation treated plants and rinsed with water to remove adhering soil. Various growth parameters, including shoot length, root length, fresh weight and number of leaves, were checked at 42 days and 84 days after sowing (DAS) from each replication.[34] The biochemical traits, such as protein and carbohydrate of leaf extracts, were determined at 42 days and 84 days DAS of inoculated and uninoculated plants. The data was statistically analyzed by using one way ANOVA followed by Tukey's HSD post hoc test.

Metabolite Profile Fingerprint of Endophytic Fungi

Fourier transforms infrared spectroscopy (FT-IR), and GC-MS were used to analyse secondary metabolite fingerprinting in ethyl acetate extract of endophytic fungi[35,36,37]

Results

Four morphologically distinct endophytic isolates were isolated from *Curcuma longa* rhizome. AVNF4 was selected for the study.

Identification of AVNF4

Based on physiological characteristics, AVNF4 is positive for amylase, cellulase, lipase, protease, urease, gelatinase, and catalase activity (Table 1). 1658 bp of 18s rRNA gene sequence was amplified from AVNF4. The pairwise sequence similarity search of the AVNF4 strain blast in ex-taxon showed that the 18s rRNA gene sequence of AVNF4 has 100% similarity with the partial 18srRNA sequence of the Aspergillus strain (gi: MK371712.1). Phylogenetic analysis based on 18s rRNA partial gene sequence revealed that AVNF4 was closely related to *Aspergillus oryzae* (Fig. 1). Based on consistent results of sequence analysis of the 18s rRNA partial gene, AVNF4 was deposited in NCBI as *Aspergillus oryzae* with Gen bank accession number PP962402.

Table 1: Physiological characterization of Fungal endophytes

Isolate	Amylase	Cellulase	Lipase	Protease	Urease	Gelatinase	Catalase
AVNF4	+++	++	++	+++	++	++	+++

+++ - High ++ - Moderate + - Low

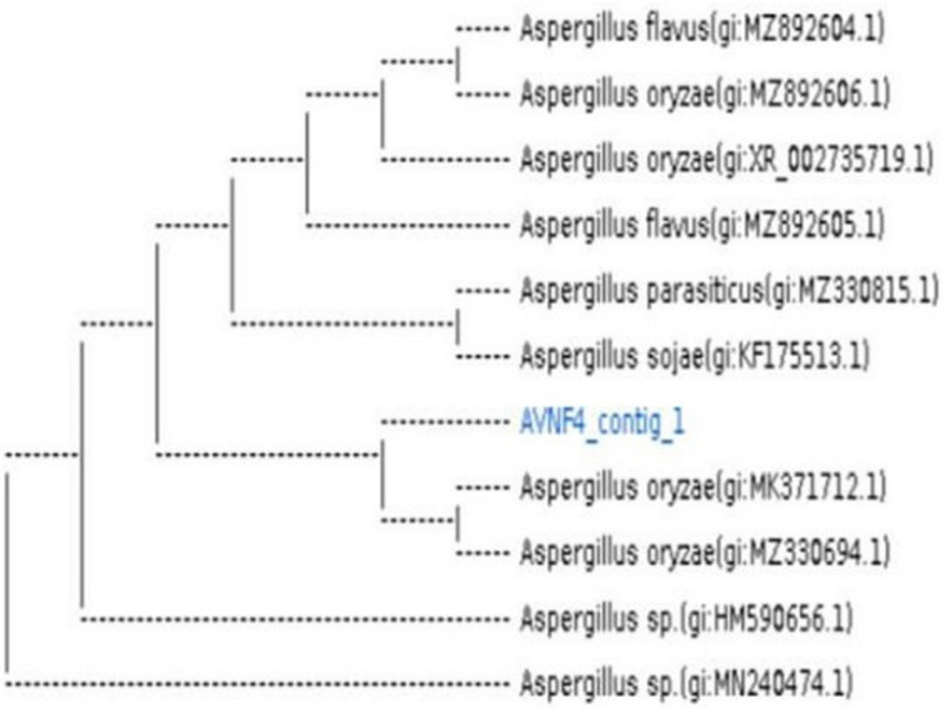

Fig. 1: Molecular identification of AVNF4

Fungal Antagonism and PGP Traits

*Aspergillus oryzae*AVNF4, screened for antagonistic activity, significantly inhibited *Fusarium oxysporum* compared to the control by reducing the hyphal growth of *F. oxysporum*, completely covering the pathogen, by overgrowing the isolate. *A. oryzae* is positive for qualitatively screening PGP traits like indole acetic acid, ammonia production, and inorganic phosphate solubilisation activities.

Effect of Physical and Chemical Parameters on the Growth of *Aspergillus Oryzae*

Aspergillus oryzae AVNF4 showed maximum growth in terms of biomass (gm/ml) at 35°C, pH 7, 1% fructose and 0.5% peptone (figure 2). *A. oryzae* showed higher values of biomass at standard culture conditions of temperature (35°C) pH 7 and nitrogen source (peptone), except carbon source where the biomass reported high in fructose. In the optimised medium, the production of IAA, ammonia and phosphate solubilisation was increased by 175%, 150% and 493%, respectively (Table 2).

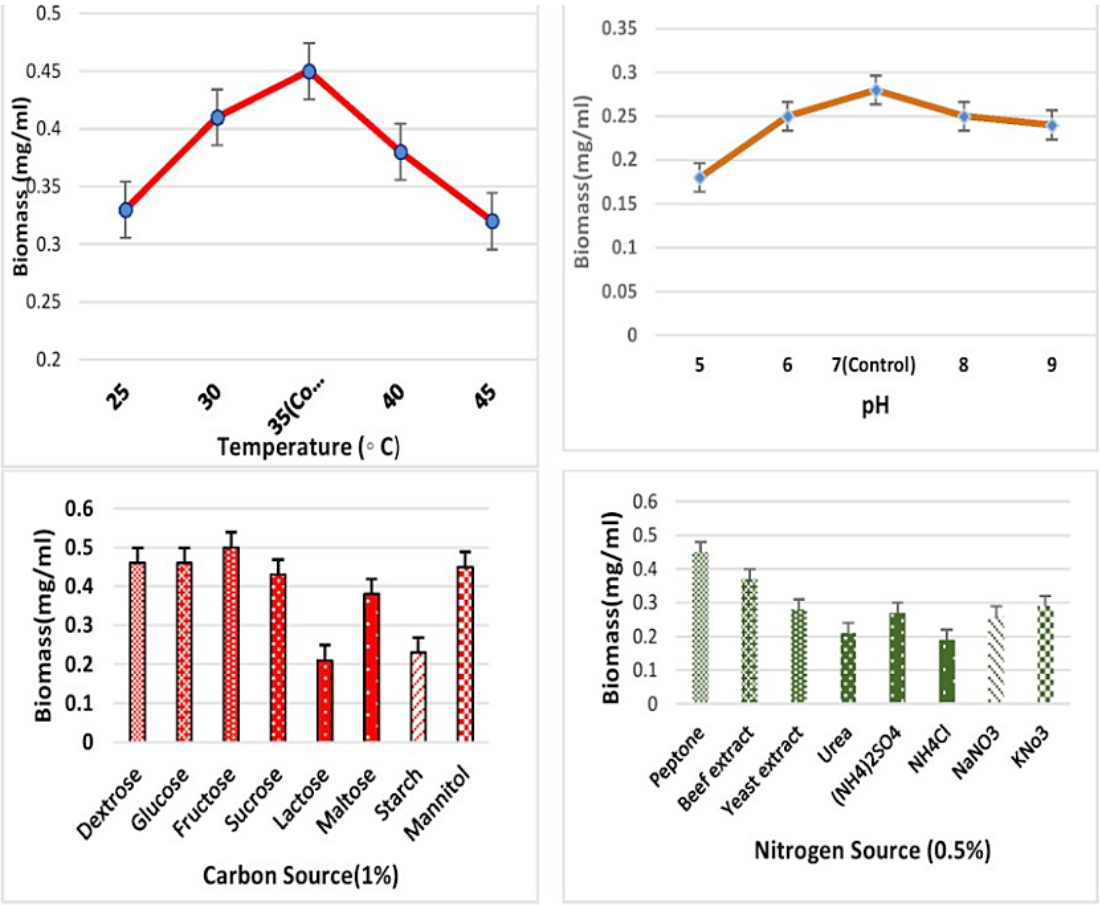

Fig. 2: Effect of physical factors (temperature and pH) and chemical factors (carbon and nitrogen) of culture medium on biomass(mg/ml) of AVNF4

Table 2: Quantitative analysis of PGP traits before and after optimization

	IAA (µg/ml)		PO_4-solubilisation(ppm)		Ammonia (µg/ml)	
	Before optimization	**After optimization**	**Before optimization**	**After optimization**	**Before optimization**	**After optimization**
Control	0.2±0.03	0.5±0.03	2±0.45	4.3±0.35	0.2±0.03	0.5±0.04
AVNF4	2.4±0.45	6.6±0.35*	125±0.45	741±3.6*	3.2±0.3	8.0±0.4*

Values are the mean of three replicates ± SE.

Greenhouse Studies

The seedling vigour index results of 48-h bacterised tomato seeds were as follows: The seedling vigour index enhanced the germination percentage (Gp), germination rate (GR), and germination index (Gi) by 70.5%, 37 %, and 76.2%, respectively, compared to the control (Table 3).

Table 3: Seedling vigour index of tomato seeds treated with Aspergillus oryzae AVNF4

Growth parameters		Control	Aspergillus.oryzae	% increase
Germination percentage (G_p)	G_p= Ni/NX100	31.83%	54.28%	70.5%
Speed of seed germination (S)	S=ni/di	Day 1=70 Day 2=20 Day 3=8.6 Day 4=5	Day 1= 50 Day 2= 60 Day 3= 32	
Germination rate (G_R)	(G_R)= seed/1st day= ……seed/nth day	103.6	142	37%
Germination index (GI)	GI=∑G/T	30.8	54.27	76.2%

The values are the mean of three replicates. ± SE, Germination percentage = the number of seeds germinated per time duration, vigour index calculated during the first week. The value in parenthesis is the percentage increase of growth parameters compared to the control.

Table 4: Plant growth promotion of tomato seedlings treated with *Aspergillus oryzae* AVNF4

	Biomass (g)		Total plant height (cm)		Shoot length (cm)		Root length (cm)		Roo/shoot ratio		No. of leaves	
	6th week	12th week	6th week	12th week	6th week	12th week	6th week	12th week	6th week	12th week	6th week	12th week
Control	0.08± 0.03	0.18± 0.04	10.6± 0.35	12.4± 0.4	9.2± 0.4	10.4± 0.4	1.4± 0.4	2.0± 0.4	0.15± 0.05	0.186 ±0.03	2± 0.5	3± 0.5
AVNF4	0.15± 0.04 (87.5%)	0.85± 0.04 (372.2%)	14.3± 0.35 (34.9%)	23.8 ±0.4 (91.9%)	12.3± 0.35 (33.7%)	20.1± 0.35 (93.2%)	2± 0.4 (42.8%)	3.7± 0.35 (85%)	0.16± 0.04 (6.6%)	0.18± 0.03 (-3.2%)	7± 0.5 (100%)	9± 0.5 (200%)

Values are mean ± SE of three replicates

Plant Growth Promotion

The activity of *A. oryzae* AVNF4 liquid bioformulation on plant growth promotion of tomatoes was studied using a pot experiment under greenhouse conditions. Growth parameters of root and shoot length and height, fresh weight, root/shoot ratio, number of leaves, and metabolites are determined in seedlings treated with *A. oryzae* liquid formulation. Readings were recorded at intervals of one week (7 days) after sowing until 12 weeks. Liquid bioformulation showed that the growth parameters are significantly increased and also showed progressive growth from the 6th to12th week(Table4)

Nutrient Metabolites

Nutrient metabolites such as reducing sugars and proteins were also increased in AVNF4 treated seedlings after 12 weeks of the treatment (Table 5). Reducing sugars has increased in the order of (100% and 233%) of AVNF4 treated plants, and protein content has increased from (77.1% and 150%) compared to untreated control.

Table 5: Primary metabolites

	Reduced Sugars		Protein	
	6th week	**12th week**	**6th week**	**12th week**
Control	0.025±0.005	0.045±0.005	0.035±0.05	0.08±0.003
A. oryzae	0.05±0.005 (100%)	0.15±0.05 (233%)	0.062±0.004 (77.1%)	0.2±0.04 (150%)

The FT-IR spectrum of the *A. oryzae* AVNF4 showed8 absorption peaks at 3322.45 cm^{-1}, 2943.98 cm^{-1}, 2831.94 cm^{-1}, 1659.17 cm^{-1}, 1450.33 cm^{-1} 1413.25 cm^{-1}, 1113.61 cm^{-1}, 1019.84 cm^{-1}, respectively, with characteristic functional groups at peak at 2943.98 cm^{-1} and 2831.94 cm^{-1} to alkyl C–H stretch, C=N bonds (1659 cm^{-1}), C-H(1413 cm^{-1}), C-O bonds (1113 cm^{-1}) andC-F stretch at 1010cm^{-1} respectively (Fig 3; Table 6).

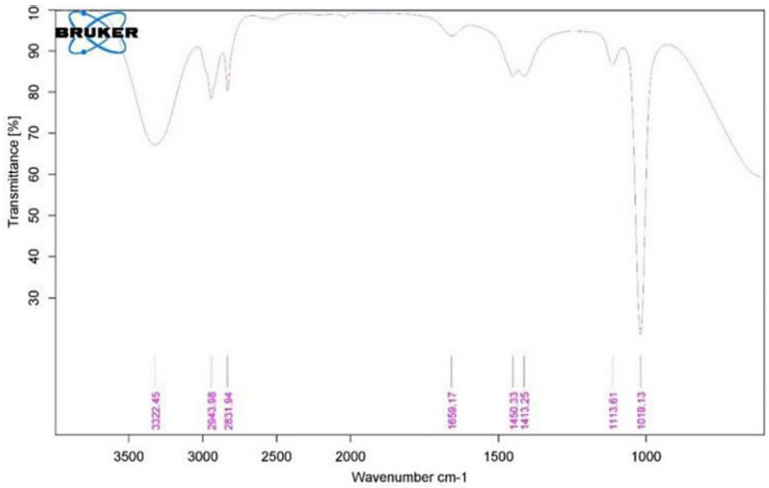

Fig. 3: Interpretation of bioactive metabolites of *Aspergillus oryzae* AVNF4 by FTIR spectral analysis

Table 6: Data Analysis by FTIR Spectra

Wavelength range:	Bond stretch and Functional groups
3322.45	Imino compounds, =N-H stretch (medium, blunt)
2943.98	C-H, (medium)
2831.94	C-H ,(weak, sharp)
1659.17	Open-chain imino (-C=N) (short)
1450.33	Methylene C-H bend (medium, sharp)
1413.25	vinyl C-H (medium)
1113.61	Alkyl-substituted ether, C-O ,(short)
1019.84	Aliphatic fluoro compounds, C-F ,(strong)

In GC MS chromatogram, Metabolic Profile of Fungal extract of *A. oryzae* AVNF4 17 bioactive metabolites "1,3-Dioxolane, 2-(1-propenyl)-, L-Prolinamide, 5-oxo-L-prolyl-L-phenylalanyl-4-hydroxy-, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, dl-Mevalonic acid lactone, Hydro cinnamic acid, Oleic acid, 9-Octadecenoic acid (Z)-, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-, 1-Allylazetidine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-Pyrrolidino-2-pyrrolidone, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methyl propyl)-, 9-Octadecenoic acid (Z)-, hexyl ester, Dehydromevalonic lactone, Acetaldehyde, (3,3-dimethyl cyclohexylidene)-, (Z)-" were observedas per the NIST Database (fig 4; Table 7)

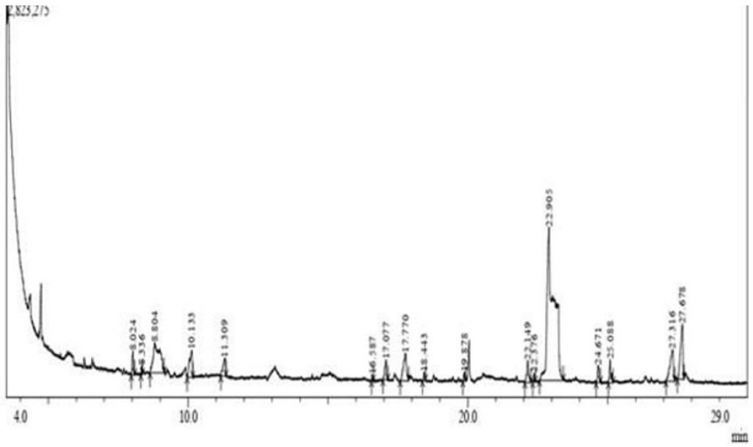

Fig. 4: GC-MS chromatogram of metabolites of *Aspergillus oryzae* AVNF4

Table 7: Metabolic Profile of Fungal extract of Aspergillus oryzae AVNF4

S. No	RT	Height (%)	Compound	Formula	Mol wt	Area(%)
1	22.905	31.72	Phenol,2,4'-isopropylidenedi	C15H16O2	228	55.32
2	8.805	6.14	1,3-Dioxolane, 2-(1-propenyl)-	C6H10O2	114	10.59
3	27.678	11.20	L-Prolinamide, 5-oxo-L-prolyl-L-phenylalanyl-4-hydroxy-	C19H24N4O5	388	6.86
4	27.316	6.45	L-Prolinamide, 5-oxo-L-prolyl-L-phenylalanyl-4-hydroxy-	C19H24N4O5	388	5.64
5	17.770	5.67	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	C7H10N2O2	154	4.69
6	10.133	5.47	dl-Mevalonic acid lactone	C6H10O3	130	3.74
7	11.309	3.66	Hydro cinnamic acid	C9H10O2	150	2.38
8	22.149	4.41	Oleic acid, 9-Octadecenoic acid(Z)-	C18H34O2	282	2.16
9	25.088	4.70	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C14H16N2O2	244	1.86
10	17.077	4.01	1-Allylazetidine	C6H11N	97	1.83
11	8.024	4.89	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144	1.49
12	24.671	3.52	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C14H16N2O2	244	1.46
13	18.443	1.72	5-Pyrrolidino-2-pyrrolidone	C8H14N2O	154	0.53
14	19.878	1.74	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methyl propyl)-	C11H18N2O2	210	0.46

15	22.376	1.79	9-Octadecenoic acid (Z)-, hexyl ester	C24H46O2	366	0.37
16	8.336	1.72	Dehydromevalonic lactone	C6H8O2	112	0.35
17	16.587	1.19	Acetaldehyde, (3,3-dimethyl cyclohexylidene)-, (Z)-	C9H14O	138	0.28

Discussion

Due to its medicinal properties and wide range of applications, the scope of research on *Curcuma longa* is leading to discoveries at the molecular and genetic levels. Endophytes help plant growth and produce bioactive secondary metabolites in disease management and agrochemical industries. Hence, *A. oryzae*AVNF4, an endophytic fungus of *Curcuma longa*, was explored to understand its impact in providing biostimulants to the growth of tomato seedlings as a biofertiliser and nutrient for sustainable tomato growth.

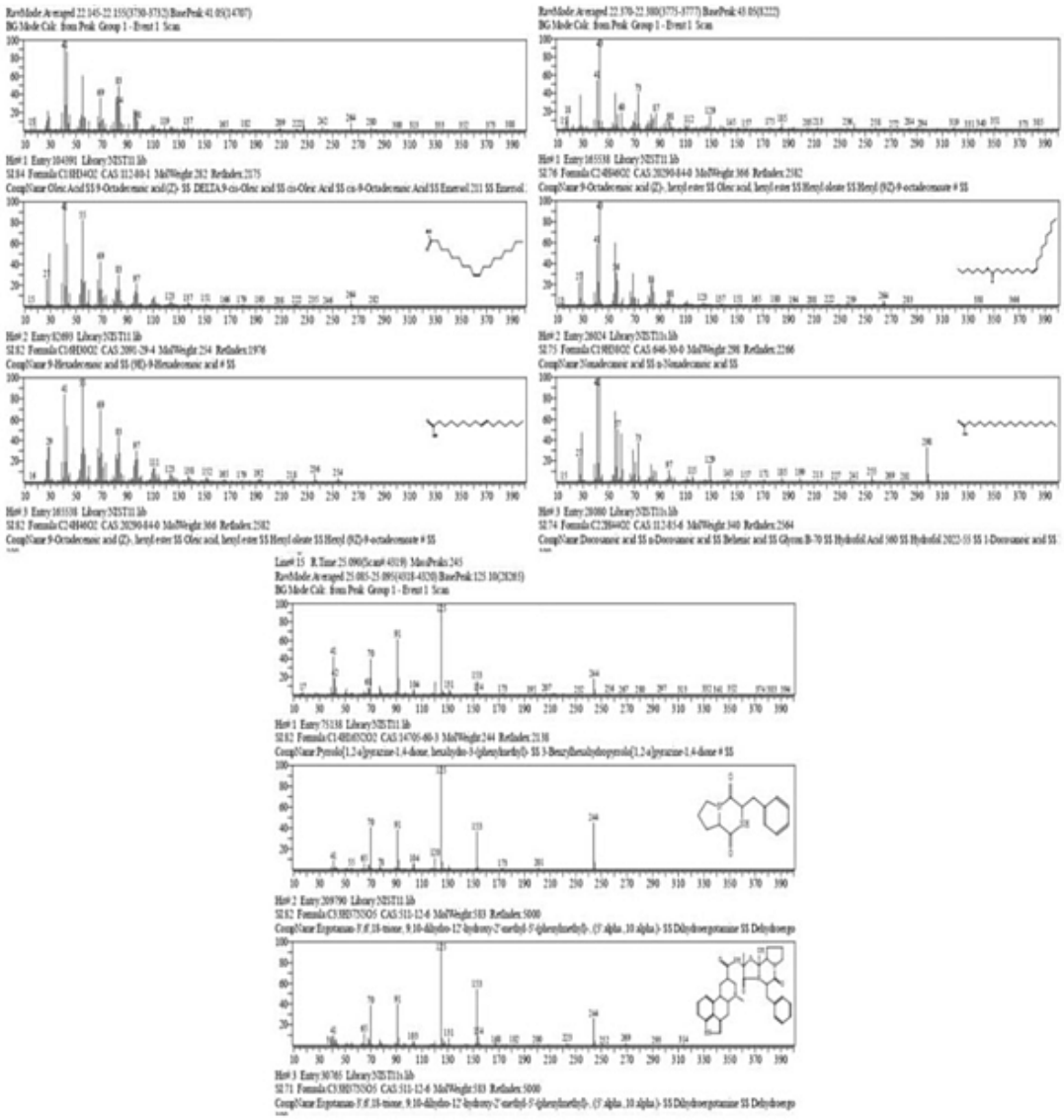

Fig. 5: Spectrum ofActive metabolites of A. oryzae AVNF4 identified in GC-MS

By producing hydrolytic enzymes such as cellulases, proteases, and pectinases that break down cell walls or plant tissue, endophytes colonise themselves inside the host plant.[38–43] Endophytic fungi isolated from oil seeds and medicinal plants exhibited significant cellulase and pectinase activity levels. Hydrolytic extracellular enzymes, specifically cellulase, protease, and pectinase, are produced by 72% of endophytes isolated from medicinal plants. These enzymes serve as bioactivity to extract nutrients from hosts, bio-resistance during pathogen-host interactions to prevent microbial pathogenic infection, and, on the other hand, to enhance the nutritional status of plants.[44] Similarly, the production of hydrolytic enzymes like amylases, cellulase, pectinases and gelatinases (Table 1) by *A. oryzae* AVNF4 is responsible for degrading the hyphal cell wall of phytopathogen. It may also be the driving force for the positive interaction of *A. oryzae* AVNF4 with the root system of tomato seedlings, resulting in a significant enhancement in the growth of tomato seedlings (Table 4). Fungal endophytes belonging to the genera *Trichoderma, Fusarium, Pestalopsis* and *Kocuriaroceawere* were reported to be isolated from rhizomes of *Curcuma longa*.[45]

Endophytic fungi, *A. favus, A. fumigatus, A. nidulans,* and *R. oryzae* isolated from *Ocimumbasilicum* plant and rhizospheric soil inhibited the growth of *F. oxysporum.*[19, 6]. Metabolic products produced from fungi have properties of antibacterial, antifungal, antidiabetic, antioxidant, and immunosuppressive. They are proven to inhibit the growth of different pathogenic fungi and bacteria.[46] A.oryzae AVNF4 arrested growth of *F. oxysporum* in dual culture assay. Similar to our results, hyphal parasitism of *P.aphanidermatum* by *Trichoderma* spp. Isolated from soil was reported.[47]. Earlier studies[45] reported that endophyte *T. harzinum* Thar DOB-31, isolated from the rhizome of *Curcuma,* inhibited the mycelial growth of *R. solani* and *P. aphanidermatum* by76.9% and 76%. Volatile compounds of endophytes were also reported to control the adverse effects of drought and salinity stresses and influence plant growth.[48,49]

Endophytes promote plant growth directly by producing hormones, including indole-3-acetic acid (IAA), gibberellins, cytokinins, phosphate solubilisation, N_2 fixation or indirectly by producing antibiotics, and siderophores.[49,50] Fungal endophytes like *Aspergillus spp., P. citrinum* and *P. oxalicum* isolated from seaweeds can phosphorus solubilising activity.[51] *A. oryzae* AVNF4 is favourable to PGP traits like IAA by producing a pink colour upon the addition of Salkowski reagent, ammonia production by the Nesslerization method and inorganic Phosphate solubilisation by forming a clear halo zone around the culture when supplemented with tricalcium phosphate. Based on the results, *A. oryzae* AVNF4 can be considered a potential isolate with multiple PGP traits.

The optimisation of physical and chemical factors in growth reveals that *A. oryzae* AVNF4 was a low-cost maintenance culture that exploited higher biomass (Fig 2). Production of PGP traits has enhanced by 175%, 150% and 493% of IAA, NH3 production and PO_4-solubilization after optimisation (Table 2), indicating that enhancement primarily relies on carbon source rather than pH, temperature and nitrogen source. Hence, a 1% fructose-enriched medium could have enhanced the growth and PGP traits of *A. oryzae* (Fig 2). Therefore, optimised media can be considered as promising liquid bioformulation. Further investigation is needed to enhance shelf life.

A. oryzae AVNF4-treated tomato seeds showed a good seed vigour index compared to *A. oryzae* untreated control seeds (Table 3). Earlier studies [52] showed that endophyte *Aspergillus oryzaeN* colonised *R. sativus* seedlings through seed inoculation and acted as plant growth promoters.

Administration of *A. oryzae* AVNF4 liquid formulation to tomato seedlings in pot experiments showed progressive enhancement of growth parameters of tomato from the sixth week to the 12th with statistically significant values. Also, it is evidenced that the *A. oryzae* AVNF4 formulation has a high potential for tomato seedling growth (Table 4). Similar to our results, it was reported that *A. oryzae* treated cacao (*Theobroma cacao*), the plant synthesised kojic acid, making the plant more tolerant to insects and pathogens.[53] Endophytes *A. tubingensis, A. alabamensis* and *A. oryzae* act as plant growth promoters and are commercially used as eco-friendly agents for the defence of pepper seedlings against *Fusarium* wilt disease.[54,55]

Among different microbial groups, fungi have been reported to be more efficient IAA production and phosphate solubilisers in comparison to bacteria influencing overall plant growth and root development, which is a direct mechanism by which biocontrol agents promote shoot and root growth and leaf area in plants.[56, 57,58] Similarly, *A. oryzae* (AVNF4) reported high amounts of IAA, ammonia production and inorganic phosphate solubilisation (Table 2). Production of growth-promoting metabolites by fungi is one of the reasons to help host plants survive under stress conditions by secreting favourable secondary metabolites. Fungal endophytes like Alternaria, *Aspergillus, Cladosporium, Colletotrichum, Fusarium, Talaromyces, Trichoderma* and *Penicillium* promote plant growth by improving soil structure and by providing resistance against biotic and abiotic stresses.[59,60] Liquid bioformulation without a carrier can be handled easily and has the properties of longer shelf life without any contamination.[61] Significant enhancement of root growth of a plant when inoculated with fungal endophytes differs with different endophytes, as endophytes have different abilities for IAA production[62]

When compared with the liquid bioformulation uninoculated control plants, there was a significant increase in fresh weight, root and shoot length, and number of leaves. Primary metabolite content, like protein and carbohydrate content, was also higher in tomato plants treated with *A. oryzae* AVNF4 liquid bioformulation, progressively from the 6th week to the 12th week (Table 4).The results revealed that liquid bioformulation of *A. oryzae* AVNF4 was promising in retaining the ability to promote tomato growth.

Plant growth-promoting bacteria like *Bacillus, Brevi bacillus, B. subtilis,* and *Paenibacillus* are used as biofertilisers for *Brassica napus, Cajanus cajan, Cicer arietinum, Vigna radiata*, and rice.[63-67] Our results provide evidence that *A. oryzae* could act as an endophyte, work as a plant growth promoter, and provide some protection against *F. oxysporum*.

In the GC MS chromatogram, a significant peak was observed at RT 22.9 with the molecular weight of 228 g/mol with the chemical formula C15H16O2. Compared to the NIST library, a significant peak with a molecular weight of 228 g/mol and the chemical formula C15H16O2 was seen at RT 22.9 in the GC MS chromatogram. The main compound was determined to be Phenol2,4'-isopropylidene, a BPA (Bisphenol A) aberration brought on by analysis compared to the NIST library. The following significant peak, with a 114 g/mol molecular weight and the chemical formula C6H10O2, was detected at RT 8.805. The FTIR data revealed that 1,3-Dioxolane, 2-(1-propenyl)- was the main compound found in the crude extract of *A. oryzae* compared to the NIST library. Similarly, it has been verified that the compounds produced by *A. oryzae* identified in the GC-MS study and those found in the FTIR analysis are the same. According to GC-MS data, the *A. oryzae* AVNF4 crude extract includes volatile organic compounds and secondary metabolites that effectively suppress phytopathogens, promote plant growth, and induce systemic resistance.[36,68, 37]

Metabolic profile of fungal extract of *A. oryzae* AVNF4 as per the NIST Database, bioactive metabolites such as 1,3-Dioxolane, 2-(1-propenyl)-, L-Prolinamide, 5-oxo-L-prolyl-L-phenylalanyl-4-hydroxy-, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, dl-Mevalonic acid lactone, Hydro cinnamic acid, Oleic acid, 9-Octadecenoic acid (Z)-, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-, 1-Allylazetidine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-Pyrrolidino-2-pyrrolidone, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methyl propyl)-, 9-Octadecenoic acid (Z)-, hexyl ester, Dehydromevalonic lactone, Acetaldehyde, (3,3 dimethyl cyclohexylidene)-, (Z)- showed bioactivities like antifungal, root colonisation, anti-inflammatory, plant defence mechanisms and promotes plant growth and metabolism.[69-77] Pyrrolo [1,2- a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) with a molecular weight of 244.2 g/mol was previously isolated from endophytic *Neotyphodium* spp. and *Epichloe* species function against phytopathogens and worms and also observed during plant-microbe relationships (Fig 5). It is also related to pyrazine derivatives and other diketopiperazine-mycotoxins, such as aspergillic acid, produced by the *Aspergillus* and *Candida* species[78-81]

In earlier studies, an endophytic fungus, *Aspergillus oryzae* YRA3, and *A. flavus*, isolated from the wild plant *Atractyliscarduus* (Forssk.) C.Chr, *E. genicu* late reported the presence of pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro, Oleic acid and

Octadeconoic acid (fig 5)involved in plant growth promotion of sorghum plants when inoculated with *A. oryzae* YRA and having biocontrol activity against phytopathogens like *Fusariumoxysporum*, *Eupenicilliumbrefeldianum*, *Alternaria phragmospora* and *A. alternate*.[54,21]

The presented data indicate the possibility of using *A. oryzae* as a biocontrol agent against the phytopathogenic fungi *F. oxysporum*. However, this requires further screening of many *A. oryzae* from different regions of India.

Conclusion

An endophytic fungus of *Curcuma longa*, *A. oryzae* AVNF4, showed positive interaction with the root system of tomato due to its physiological characteristics (production of hydrolytic enzymes), resulting in the significant enhancement in the growth of tomato seedlings. For the first time, an attempt was made to develop a liquid bioformulation of *A. oryzae*, an endophyte of turmeric. Growth optimisation of *A. oryzae* has also enhanced PGP traits after optimisation of culture conditions and responded positively to stimulate the growth of tomato seedlings. The growth promotion of tomato seedlings in pot experiments has confirmed the potential of liquid formulation as a promising biofertiliser and bio-nutrient for the sustainable growth of tomatoes. Hence, this liquid formulation will be a step in the initiation of the development of an environmentally safe and non-deleterious liquid biofertiliser for vegetable crops.

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Conflict of Interest

Authors do not have any conflict of Interest

Data Availability Statement

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval

Author contributions

- **S. Narmada:** Data Collection, Methodology, Writing – Original Draft.
- **Amrutha V Audipudi:** Conceptualization, Analysis, Writing,Review & Editing.

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