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RESEARCH ARTICLE

Deciphering the antifungal activity of *Lentinus squarrosulus* against *Fusarium* wilt in tomato

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Abstract

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *Lycopersici*, is a major soil-borne disease threatening tomato (*Solanum lycopersicum*) production worldwide, causing yield losses of up to 80 %. This study evaluated the antifungal potential of the medicinal mushroom *Lentinus squarrosulus* against FOL, aiming to identify bioactive metabolites for sustainable disease management. FOL was isolated from infected tomato plants in Coimbatore, identified morphologically and molecularly (GenBank accession PQ350410) and used for *in vitro* assays. Nine macro basidiomycetes were screened via dual culture assay; *L. squarrosulus* TNAU L3 showed the highest mycelial growth inhibition (55.72 %), followed by *Auricularia auricular* (49.26 %) and *L. squarrosulus* TNAU L2 (43.74 %). Ethyl acetate extracts of *L. squarrosulus* significantly inhibited *F. oxysporum* f. sp. *lycopersici* growth in agar well diffusion assays, with inhibition increasing from 37.44 % at 500 ppm to 67.53 % at 2000 ppm. GC-MS analysis of extracts revealed antimicrobial compounds including eicosatrienoic acid, benzofuran, isopimaric acid and cyclododecanol, linked to antifungal and immunomodulatory activities. Pathway analysis indicated these metabolites are synthesized via the pentose phosphate pathway, contributing to both direct pathogen suppression and enhancement of plant defense through metabolic modulation. The dual mechanism-pathogen inhibition and host immunity activation-suggests *L. squarrosulus* as a promising eco-friendly biocontrol agent. This work highlights the potential of macrobasidiomycete-derived metabolites as alternatives to synthetic fungicides and provides a preliminary framework for developing natural fungicidal formulations. Further field validation and optimization of metabolite application are needed to advance integrated management strategies for Fusarium wilt in tomato.

Keywords: bioactive metabolites; *F. oxysporum f. sp. lycopersici*; *L. squarrosulus*; mycelial growth

Introduction

Tomato (*Solanum lycopersicum* L.) of *Solanaceae* family which has its origin in western South America, is a herbaceous crop. As per FAO 2019 data, more than 5 million hectares were covered by tomato production, highlighting its global significance. Around one -fifth of the total production was shared by the following countries- China, India, Turkey, the United States and Egypt. However, market demands have led to the creation of monoculture conditions, which significantly promote the emergence and recurrence of various diseases and jeopardize the quantity and quality of agricultural output. Tomato crops can suffer yield losses ranging from 20 % to 80 % as a result of diseases (1).

Fusarium wilt is a soil-borne disease averting tomato production (2, 3). It causes yield loss of up to 10 %-80 %, which endangers the livelihood of small-scale farmers (4). The pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* is the causative organism for that disease. Its place of entry is through the roots of the host, from where it conquers the vascular tissues and affects nutrients and water intake (5). One plant starts to wilt from one side starting from the older leaves and then covering the entire plant (6, 7). Furthermore, in the cross sections of the stem tissue, stem degradation and vascular discoloration will be observed, particularly in damp conditions. Controlling the disease spread is challenging because it forms resting structures called chlamydospores that can endure in soil for ten years (8).

Chemical usage and its detrimental effects on environment, humans and animals are alarming and so its usage has to be limited. Chemical management mainly relies on seed treatments with fungicides such as captan and thiram, soil drenches using systemic fungicides like carbendazim, benomyl and thiophanate-methyl and triazoles including tebuconazole or propiconazole, while traditional soil fumigants like methyl bromide or metam sodium have largely been restricted due to environmental concerns (9). Biological methods are sustainable and environmentally friendly strategies, which are essential in alleviating those detrimental effects. Biological management emphasizes the use of antagonistic

microorganisms such as *Trichoderma spp.*, *Pseudomonas fluorescens* and *Bacillus subtilis*, which suppress the pathogen through competition, antibiosis and induction of systemic resistance. Additionally, organic amendments like neem cake, compost or farmyard manure, along with arbuscular mycorrhizal fungi, improve soil health, enhance plant vigor and reduce the severity of Fusarium infection (10).

Among the biological control measures, one of the untapped natural bio resources with a vast scope is mushrooms. Multiple bioactive metabolites, including anthraquinones, alkaloids, anthrones, coumarins, fatty acids, phenols, saponins and steroids have been extracted from mushrooms, according to scientific research (11-13). These metabolites exhibit antiviral, antifungal, antibacterial and antioxidant effects, which prove their usage in biocontrol of pathogens (14). Apart from the above listed properties, mushrooms also play the role of tyrosinase inhibition and plant growth promotion (15). Although mushrooms are used for medicinal and nutritional purposes, they also hold promise for sustainable agriculture as a source of secondary metabolites that have anti-plant pathogen inhibitory properties (16, 17). Despite several studies on mushroom bioactives, limited information is available on their role in Fusarium wilt management in tomato, particularly focusing on *Lentinus squarrosulus*. Therefore, this study focuses on identifying the bioactive compounds of *L. squarrosulus* against Fusarium wilt, which aids in the creation of fungicidal formulations using naturally available macro basidiomycetes.

Materials and Methods

Isolation of pathogen

Fusarium infested tomato root samples were collected from Jagirnaickenpalayam region of Coimbatore. Twenty plants per plot were randomly collected and assessed for *Fusarium* wilt. Infected root tissues were sliced into small pieces, followed by surface sterilization for one minute with 1 % sodium hypochlorite. Subsequently washed with sterile distilled water three times and allowed to air-dry. Using the half-plate technique, the root bits were then placed in sterile Petri dishes filled with potato dextrose agar (PDA) medium and incubated at room temperature (25 ± 2 °C). Hyphal tip culture method was employed for obtaining pure culture of the pathogen (18). Furthermore, pure culture of the pathogens was preserved separately on PDA slants in test tubes and secured under refrigerated conditions for subsequent studies (19). Morphological characters such as mycelial characteristics, spore morphology, size and shape of the conidia were observed through microscopic examination. Molecular characterization was confirmed using the universal primer pair ITS1/ITS4 (5'-TCCGTAGGTGGACCTGCGG-3'/5'-TCCTCCGCTTATTGATATGC-3') obtained from NCBI Database.

Collection of macrobasidiomycetes

For the research, some of the medicinal mushrooms cultures like *Ganoderma lucidum* TNAU G1, *G.lucidum* TNAU G3, *Schizophyllum commune* TNAU S1, *S. commune* TNAU S2, *L. squarrosulus* TNAU L2, *L. squarrosulus* TNAU L3, *Gymnophilus junonius* TNAU GY, *Agrocybe aegerita* TNAU AG 1 and *Auricularia auricular* TNAU A1 were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. All these isolates were conserved in PDA medium at 25 °C and periodically subcultured in order to use for further experiments.

Dual culture assay

The efficacy of obtained medicinal mushrooms to restrict the mycelial growth of *F. oxysporum* f. sp. *lycopersici* was observed under *in vitro* conditions using dual culture assay. From the rapidly expanding margins of 10 days old medicinal mushroom cultures, a 6 mm mycelial disc was cut using sterile cork borer and was placed on a plate containing sterile PDA (Potato dextrose agar) medium. In the similar way, mycelial disc of *F. oxysporum* f. sp. *lycopersici* was placed in the plate at the end opposite to that in which the disc of mushroom was placed. As controls, Petri plates that were only cultured with *F. oxysporum* f. sp. *lycopersici* were employed. The pathogen growth was measured up to 10 days after inoculation. The plates were maintained at 28 ± 2 °C and the treatments were replicated three times with CRD as the experimental design. The percentage (%) of mycelial growth inhibition was calculated in order to evaluate the inhibition induced by medicinal mushrooms, using the formula given below (20).

$$\text{Per cent inhibition over control (\%)} = \frac{C - T}{C} \times 100 \quad \text{(Eqn. 1) (20)}$$

Where, C is the growth of pathogen in control (mm) and

T is the growth of pathogen in dual culture plate (mm)

Bioprospecting of macro basidiomycetes for bioactive compounds

Based on the dual culture assay, *L. squarrosulus* was used to assess the inhibitory potential against *F. oxysporum* f. sp. *lycopersici*. Seven days old, 5 mm-diameter mycelial discs of the mushroom fungus *L. squarrosulus* were obtained from the colony margin. Furthermore, the mycelial discs were inoculated into 250 mL conical flasks, containing 100 mL of Potato Dextrose broth. The flasks were incubated at 25 °C and agitated at 120 rpm for 20 days in an incubator cum rotary shaker. Following the collection of the culture filtrate, the mycelial mat was separated using Whatman No. 40 filter paper. Additionally, the culture filtrate was centrifuged for 15 minutes at 10,000 rpm and the cell-free culture filtrate was extracted consecutively using ethyl acetate solvent (1:1 v/v). For the same solvent, liquid-liquid extraction was performed four times. To acquire the residues, the solvent extract was separately evaporated using a rotary evaporator at a lower pressure. Upon drying, 1 mg of the extract was obtained, which was then suspended in methanol (1 mg/mL). The solvent extract condensate was filtered through a membrane filter (0.2 µm) and kept at 4 °C for further research (21).

Antimicrobial activity of ethyl acetate extracts of macro basidiomycetes under *in vitro* conditions

Using the agar well diffusion method, the ethyl acetate fraction of the cell-free culture filtrate of *L. squarrosulus* was evaluated against the mycelial growth of *F. oxysporum* f. sp. *lycopersici* at various concentrations, namely 500, 1000, 1500 and 2000 ppm (22). Sterile Petri dishes were filled with PDA medium, which was then set aside to solidify. On solidification, a sterile cork borer was used to make 5 mm diameter wells on each of the four corners, leaving 1 cm between each corner and the periphery. 100 µL of the various amounts of solvent-extracted metabolites were added to each well. A 5 mm mycelial disc of *F. oxysporum* f. sp. *lycopersici*, harvested from a seven days old culture was then

placed in the middle of each Petri dish and cultured for seven days at 28±2 °C. Both water and methanolic control were maintained. Observations on the percent inhibition of mycelial growth of pathogens were taken (20).

$$\text{Per cent inhibition over control (\%)} \frac{C - T}{C} \times 100 \quad \text{(Eqn. 2) (20)}$$

Where, C is the growth of pathogen in control (mm) and

T is the growth of pathogen in dual culture plate (mm)

Metabolic insights into antagonist-pathogen dynamics

To identify the non-volatile organic components or metabolites that inhibit *F.oxysporum* f. sp. *lycopersici* cell-free culture filtrates were obtained from *L. squarrosulus*. To ascertain how the antagonist and pathogen interact, PDB broth (100 mL) was inoculated with *F. oxysporum* f. sp. *lycopersici* and *L.squarrosulus*. PDB broth (100 mL) containing only the pathogen or antagonist was maintained as the control broth. Since metabolite production reaches its peak after 10 to 15 days of incubation, 15 days old culture was taken for metabolite extraction. After 15 days of incubation at 28 °C, the cultures were filtered through two layers of filter paper. The extracted culture filtrate was combined with an equal volume of ethyl acetate and the combination was kept overnight in a shaker at 150 rpm. A separating funnel was then used to separate the ethyl acetate component. A rotary evaporator was used to evaporate and dry the collected upper phase. The dried residue was dissolved in 1 mL of HPLC-grade methanol, which was used to perform GC-MS analysis. The instrument was set up as follows: the injector port temperature was set at 250 °C, the interface temperature was set at 250 °C and the source was kept at 200 °C. The oven temperature was programmed to be 70 °C for two minutes, 150 °C at 8 °C/min and up to 10 °C/min at 260 °C. The split ratio was set to 1:50. The non-polar DB-35 MS column was used and its dimensions were 0.25 mm OD × 0.25 µm ID × 30 m in length. Helium was used as the carrier gas at a rate of one mL per minute. From 50 to 550 Da, the MS was scheduled to scan. The source was kept at 200 °C and motor vacuum pressure was maintained below $4.5e^{-6}$. -70eV was the ionization energy. Additionally, the MS included a built-in pre-filter that decreased the neutral particles. NIST4 and WILEY9, two built-in libraries in the data system, each include over five million references for spectrum matching and search. Pathway analysis was performed using Cytoscape v3.3 with the KEGG database for mapping gene interactions.

Statistical analysis

The experimental data were statistically analysed using the Completely Randomized Design (CRD) as outlined earlier (23). All treatments were replicated three times and arranged randomly to ensure unbiased estimation of treatment effects. The data were analysed using R software version 2.14.1. Prior to analysis, the assumptions of ANOVA, including normality of residuals and homogeneity of variances, were tested and satisfied. Analysis of variance (ANOVA) was then employed to test the significance of treatment differences and mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$, following significant ANOVA results.

Results

Isolation and identification of pathogen

F. oxysporum f. sp. *lycopersici* was isolated from Coimbatore region, purified and maintained on PDA medium. Morphological characterization revealed pinkish violet colony on the upper side of the plate. Similarly, under microscopic observations, hyaline, septate mycelium, microconidia and macroconidia were observed. The microconidia were hyaline, oval-shaped and either unicellular or bicellular, with an average size of 14.01 µm in length and 3.95 µm in width. The macroconidia were slightly curved, hyaline and showed 2-3 septations, measuring approximately 18.20 µm in length and 4.87 µm in width, which were used for confirming the pathogen as *Fusarium* (Fig. 1). Amplicon size of ~ 560bp band was obtained which confirmed *F. oxysporum* f. sp. *lycopersici* through ITS primers and sequence homology was performed using BLAST which has shown 99 % sequence homology with reference strains. The sequence was submitted to GenBank and accession number PQ350410 was obtained. This isolate was used for other experiments.

Fig. 1. Pure culture and microscopic view of *F. oxysporum f. splycopersici.*

Dual culture analysis for testing the efficacy of macro basidiomycetes against *F. oxysporum f. sp. lycopersici*

To determine the ability of various macro basidiomycetes against *F. oxysporum* f. sp. *Lycopersici* dual culture assay was performed with three replicates for each treatment. All the tested macro basidiomycetes exhibited significant reduction in the mycelial growth of the pathogen, amongst which *L. squarrosulus* TNAU L3 has shown highest mycelial inhibition of 55.72 % (Table 1 and Fig. 2), followed by *A. auricular* TNAU A1, *L. squarrosulus* TNAU L2, *G. lucidum* TNAU G3, *S. commune* TNAU S1, *S. commune* TNAU S2, *G. lucidum* TNAU G1 and *A. aegerita* TNAU AG1 showing 49.26 %, 43.74 %, 41.56 %, 33.54 %, 32.34 %, 28.31 % and 21.86 % growth inhibition respectively. Since the growth of *G. junonius* was very slow, it exhibited no inhibition on the growth of *F. oxysporum* f. sp. *lycopersici.* Consequently, *L. squarrosulus* TNAU L3 was utilized for further analysis.

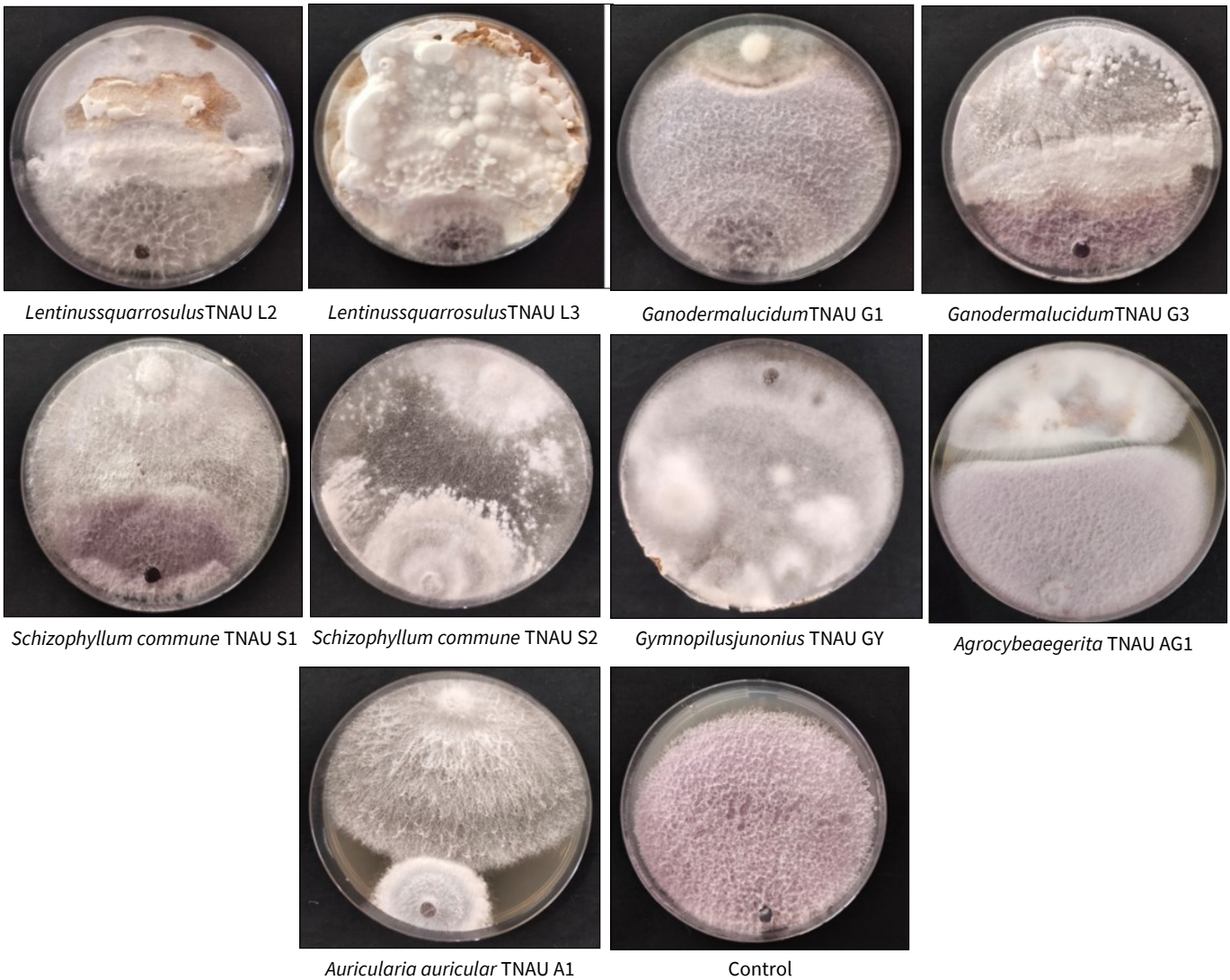
Antimicrobial activity of ethyl acetate extracts of *L. squarrosulus* under *in vitro* conditions

The ethyl acetate extracts of *L. squarrosulus* were extracted by inoculating it in PD Broth. Various concentrations such as 500, 1000, 1500 and 2000 ppm were made to test the concentration for inhibiting the mycelial growth of *F. oxysporum* f. sp. *lycopersici*. Percent inhibition ranged from 37.44 % to 67.53 %. Maximum inhibition was observed at 2000 ppm with percent inhibition of 67.53 % (Table 2 and Fig. 3).

Table 1. Screening the antagonistic activity of mushroom fungi against *F. oxysporum* f. sp. *lycopersici*

S.No	Treatment	*Mean mycelial growth (in mm)	Percent inhibition over control
1.	*Lentinus squarrosulus* L3	39.85^{a} (39.16)	55.72
2.	*Auricularia auricular*	45.67^{ab} (42.53)	49.26
3.	*Lentinus squarrosulus* L2	50.63^{bc} (45.38)	43.74
4.	*Ganoderma lucidum* G3	52.60^{c} (46.51)	41.56
5.	*Schizophyllum commune* S1	59.81^{d} (50.68)	33.54
6.	*Schizophyllum commune* S2	60.89^{d} (51.31)	32.34
7.	*Ganoderma lucidum* G1	64.52^{de} (53.46)	28.31
8.	*Agrocybe aegerita*	70.33^{e} (57.02)	21.86
9.	*Gymnopilus junonius*	90.00^{f} (71.60)	0.00
10.	Control	90.00^{f} (71.60)	0.00
	CD ($p \leq 0.05$)	6.396	-
	SEd	3.066	-

*Values are the mean of three replications. Means followed by a common letter are not significantly different at 5 % level by DMRT Values in parentheses are arcsine-transformed values.



*Lentinussquarrosulus*TNAU L2 | *Lentinussquarrosulus*TNAU L3 | *Ganodermalucidum*TNAU G1 | *Ganodermalucidum*TNAU G3

Schizophyllum commune TNAU S1 | *Schizophyllum commune* TNAU S2 | *Gymnopilusjunonius* TNAU GY | *Agrocybeaegerita* TNAU AG1

Auricularia auricular TNAU A1 | Control

Fig. 2. Dual culture assay of macro basidiomycetes on mycelial growth of *F. oxysporum f. splycopersici.*

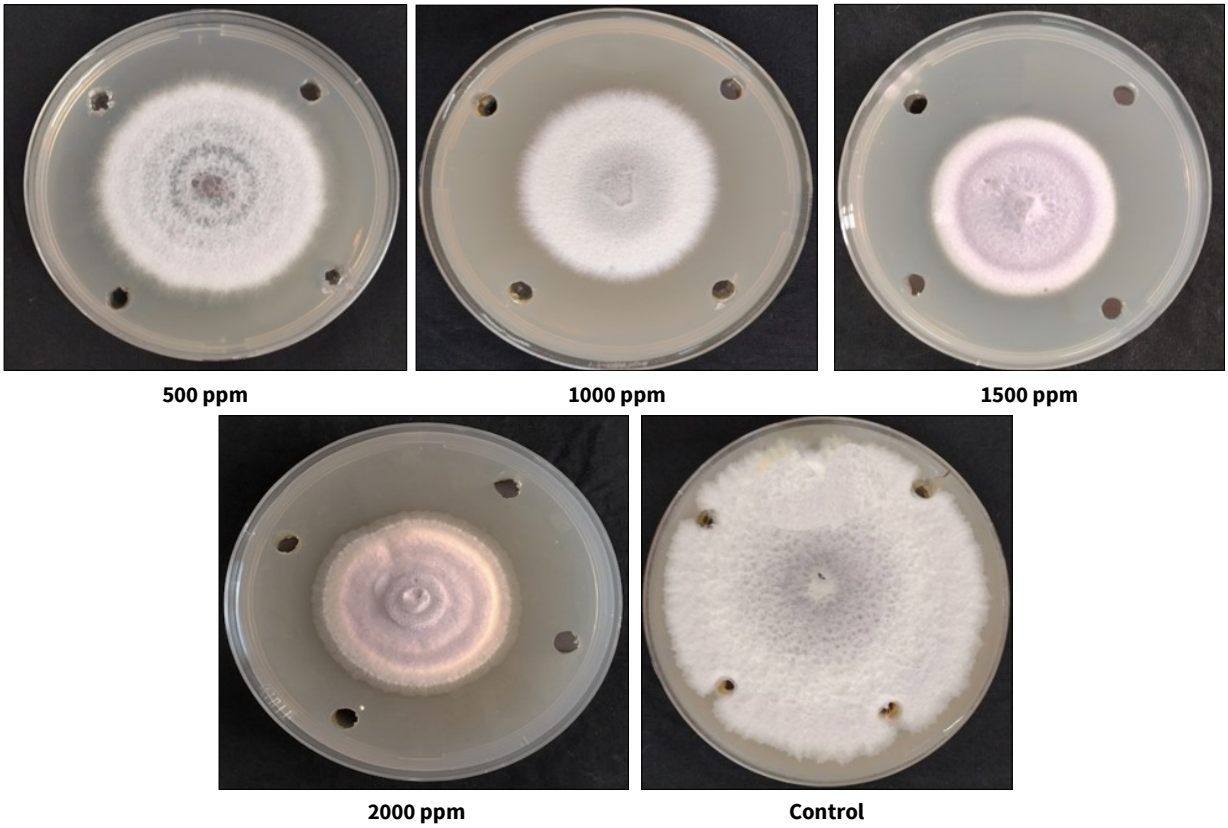


Fig. 3. Solvent fraction of *L. squarrosulus* on mycelial growth of *F. oxysporum f.splycopersici.*

Table 2. Testing the ethyl acetate extracted antimicrobial compound of *L. squarrosulus* on mycelial growth of *F. oxysporum* f. sp. *lycopersici* by agar well diffusion assay

Concentration (ppm)	*Mean mycelial growth (in mm)±SE	Percent inhibition over control
500	56.30^{d}±0.81 (48.64)	37.44
1000	48.69^{c}±0.18 (44.27)	45.90
1500	39.75^{b}±0.80 (39.11)	55.83
2000	29.22^{a}±1.17 (32.74)	67.53
Control	90.0^{e}±2.62 (71.60)	0.00
CD (p ≤ 0.05)	5.333	-
SEd	2.393	-

*Values are the mean of three replications. Means followed by a common letter are not significantly different at 5 % level by DMRT Values in parentheses are arcsine-transformed values.

Metabolic insights into *L. squarrosulus* and *F. oxysporum f. sp. lycopersici* dynamics

Antimicrobial compounds present in ethyl acetate extracts of *L. squarrosulus* were characterized through GC-MS analysis. The NIST library was utilized for validating their mass spectrum. Eicosatrienoic acid, dodecane, heptadecyn, benzofuran, isopimaric acid, spiro[azulene-1(4H),2'-[1,3]dioxolan]-4-one, octahydro-7-methyl, 1,2,4-Triazolo[2,3-a]quinazolin-2-amine, 4,5,6,7,8,9-hexahydro-5,5 pentamethylene, 2'-Hydroxy-à-naphthoflavone, 2-Methyl-4-(4-methyl-1-piperazinyl)-4-oxo-2-butenoic acid, L-Alanine, N-L-alanyl, 9,12-Octadecadienoic acid, Pinocamphone and cyclododecanol were some of the compounds possessing maximum antimicrobial activity (Area %, Retention time, Molecular weight of the listed compounds are given in Table 3). Eicosatrienoic acid was detected at 31.145 RT, possessing antimicrobial activity, has showed the highest peak area. Using cytoscape 3.3 pathway maps were generated, for assessing the key pathways involved in the interaction between *L. squarrosulus* and *F. oxysporum* f. sp. *lycopersici.* Fig. 4 shows the significant compounds and synthesis of these compounds by Pentose phosphate pathway cycle.

Table 3. Compounds obtained on GC-MS analysis of *L. squarrosulus* extracts

S.No	Compound	Peak area	Retention time	Molecular weight(g/mole)
1.	Eicosatrienoic acid	0.940	31.145	306.48
2.	Dodecane	0.547	27.738	170.33
3.	Heptadecyn	1.091	22.391	240.47
4.	Benzofuran	1.633	7.700	118.13
5.	Isopimaric acid	0.648	19.570	302.45
6.	Spiro[azulene-1(4H),2'-[1,3]dioxolan]-4-one, octahydro-7-methyl	1.529	25.927	224.30
7.	1,2,4-Triazolo[2,3-a]quinazolin-2-amine,4,5,6,7,8,9-hexahydro-5,5 pentamethylene	1.209	20.445	259.36
8.	2'-Hydroxy-à-naphthoflavone	2.533	12.207	288.30
9.	2-Methyl-4-(4-methyl-1-piperazinyl)-4-oxo-2-butenoic acid	2.123	3.073	212.25
10.	L-Alanine, N-L-alanyl	0.949	6.590	160.17
11.	9,12-Octadecadienoic acid	0.587	11.562	280.45
12.	Pinocamphone	0.562	11.842	152.23
13.	Cyclododecanol	1.488	24.577	184.32

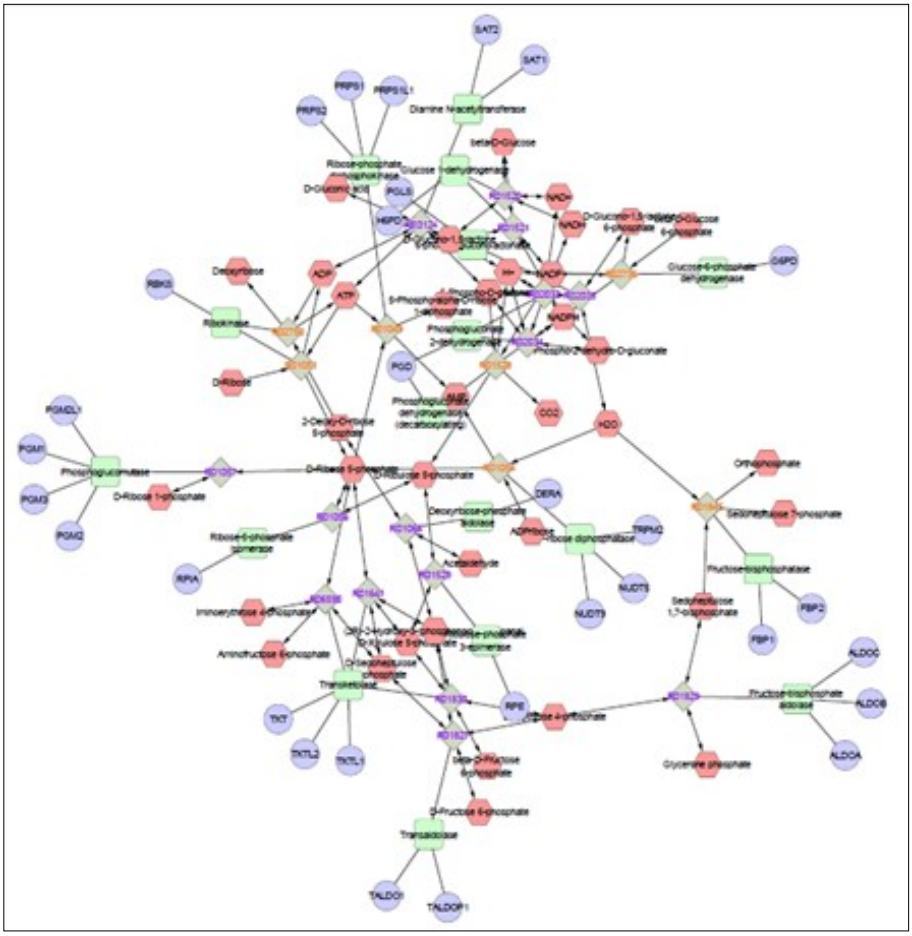


Fig. 4. Pathway analysis generated during the interaction between *L. squarrosulus* and *F. oxysporum f. sp lycopersici.* Enzymes involved in Pentose Phosphate Pathway are given in green.

Pathway analysis indicated that the metabolites of *L. squarrosulus* modulate plant immunity by influencing hormone signalling, enhancing pentose phosphate pathway cycle flux and promoting the biosynthesis of defense-related compounds. The metabolic interaction map of Pentose phosphate pathway revealed a highly interconnected network involving key enzymes, metabolites, cofactors and related pathways. The pathway was broadly classified into two functional segments, oxidative and non -oxidative phases, each playing distinct roles in cellular metabolism. In oxidative phase, glucose-6-phosphate was oxidized by glucose-6-phosphate dehydrogenase (G6PD), resulting in the formation of 6-phosphoglucono-δ -lactone and the simultaneous production of NADPH. This step was followed by the action of 6-phosphogluconolactonase and 6-phosphogluconate dehydrogenase, leading to the generation of ribulose-5-phosphate and a second molecule of NADPH, along with CO_2. These reactions represent the primary redox-active segment of the pathway, contributing to cellular antioxidant defense and biosynthetic reducing power.

The non-oxidative phase showed a series of sugar interconversions catalysed by enzymes such as transketolase, transaldolase and ribose-5-phosphate isomerase. Ribulose-5-phosphate was converted to ribose-5-phosphate (a precursor for nucleotide biosynthesis) and xylulose-5-phosphate, which further participated in reactions yielding glyceraldehyde-3-phosphate (G3P) and fructose-6-phosphate (F6P). These metabolites were fed back into glycolysis, indicating tight regulation and interaction between catabolic and anabolic processes. Additionally, the network map illustrated extensive crosstalk with glycolysis, the tricarboxylic acid (TCA) cycle and amino acid biosynthetic pathways. The presence of shared intermediates such as G3P and F6P emphasized the flexibility of the PPP in adapting to varying metabolic demands. Enzymatic nodes such as G6PD and transketolase were observed as central hubs, regulated through feedback by cofactors like NADPH and ATP, whose concentrations influence pathway flux. The map also highlighted several biosynthetic linkages, particularly involving PRPP (phosphoribosyl pyrophosphate) synthesis from ribose-5-phosphate, which is essential for purine and pyramidine nucleotide production. Furthermore, connections with aromatic amino acid biosynthesis via erythrose-4-phospahte (E4P) were evident. This suggests a dual mode of action: direct pathogen inhibition and indirect activation of host defense mechanisms.

Discussion

Macro basidiomycetes are increasingly recognized not only for their pharmacological and nutraceutical value but also for their promising role in sustainable agriculture (24). Despite the wide-ranging biological activities, their potential as biocontrol agents in plant disease management has remained largely underexplored until recent years. In the present study, *L. squarrosulus* TNAU L3 demonstrated significant antagonistic activity against *F.oxysporum* f. sp. *lycopersici,* a major soil-borne pathogen of tomato. The dual culture assay revealed mycelial growth inhibition ranging from 21.86 % to 55.72 %, with the highest inhibition observed in *L. squarrosulus* TNAU L3, suggesting its potent antifungal capability. Comparable results were reported in earlier studies, where wild macrobasidiomycete *Coprinellus disseminates*, *Marasmiellus*

palmivorus, *Trametes maxima* and *Lentinus sajor-caju* effectively suppressed the growth of rice fungal pathogens *Curvularia lunata*, *Bipolaris panici-miliacei* and *Nigrospora* sp., highlighting the broader spectrum antifungal potential of macro basidiomycetes (25). The inhibitory action observed is likely due to both volatile organic compounds (VOCs) and non-volatile metabolites such as pinocamphone (26), triazoloquinazolines (27) and Spiro[azulene-1 (4H),2'-[1,3]dioxolan]-4-one, octahydro-7-methyl (28) that disrupt pathogen growth, as supported by similar studies (29).

In the current study, ethyl acetate extracts of *L. squarrosulus* showed up to 67.53 % inhibition at 2000 ppm, substantiating its strong fungistatic properties. A similar trend was reported earlier, where ethyl acetate extracts of *G. lucidum* significantly inhibited the growth of *Colletotrichum gloeosporioides* at 1 % concentration. GC-MS profiling of *L. squarrosulus* extracts revealed the presence of bioactive antimicrobial compounds such as eicosatrienoic acid, benzofuran, isopimaric acid and cyclododecanol, which are known for their antifungal and immunomodulatory properties ((17, 30). These findings align with the work, who demonstrated that Octan-3-one produced by *L. edodes* effectively inhibited the growth of *Alternaria brassicola* (31). Metabolite profiling from earlier studies also revealed similar antimicrobial agents in *L. squarrosulus,* including hexadecanoic acid ethyl ester, 9,12-Octadecanoic acid and ethyl oleate, which further reinforces its biocontrol potential (30). These secondary metabolites not only inhibit pathogens directly but also prime host defences. Studies have shown the upregulation of defense genes such as pathogenesis-related protein-1, lipoxygenase, thaumatin-like proteins when treated with metabolites (32).

Pathway analysis indicated that the metabolites of *L. squarrosulus* modulate plant immunity. The PPP is functionally divided into oxidative and non-oxidative branches, both of which play critical roles in balancing cellular metabolism and defense responses. In the oxidative phase, oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase (G6PD) generates NADPH, a central reducing equivalent required for reactive oxygen species (ROS) detoxification and for driving reductive biosynthesis of defense metabolites such as phenolics and phytoalexins (33). The additional production of ribulose-5-phosphate not only sustains nucleotide biosynthesis but also supports rapid DNA/RNA turnover during stress signalling (34). In the non-oxidative phase, sugar interconversions catalyzed by enzymes such as transketolase and transaldolase provide ribose-5-phosphate for nucleotide synthesis and erythrose-4-phosphate for aromatic amino acid biosynthesis, the latter serving as precursors for secondary metabolites (e.g. salicylic acid, flavonoids, lignin) known to strengthen plant structural and biochemical defenses. Crosstalk of PPP intermediates with glycolysis, the TCA cycle and amino acid metabolism reflects a metabolic reprogramming that integrates energy production with biosynthetic demands during pathogen challenge. The identification of G6PD and transketolase as key regulatory nodes underscores their importance in maintaining redox balance through NADPH supply and in channeling carbon skeletons toward defense-related metabolites (35). Thus, activation of the PPP by *L. squarrosulus* metabolites suggests a dual mechanism-direct inhibition of pathogen growth through metabolic interference and indirect reinforcement of host immunity by boosting NADPH-dependent antioxidant systems and secondary metabolite biosynthesis.

Supporting this, previous studies reported that *Trichoderma* sp., a well-established biocontrol agent, also modulates plant metabolism and stress responses by maintaining cellular homeostasis and releasing antifungal compounds, thereby enhancing energy mobilization during antagonistic interactions (36). The parallelism between *L. squarrosulus* and *Trichoderma* sp. suggests that macro basidiomycetes may similarly serve as eco-friendly efficient agents in integrated disease management strategies. Unlike *Trichoderma*, which is extensively studied and widely used as a biocontrol agent, *Lentinus squarrosulus* remains relatively less explored. However, it represents a promising alternative, as it produces a distinct repertoire of bioactive metabolites that may contribute to plant defense through mechanisms different from conventional fungal biocontrol agents. Collectively, these findings underscore the immense potential of macro basidiomycetes, particularly *L. squarrosulus*, as a biocontrol agent capable of suppressing phytopathogens while simultaneously enhancing host resistance through metabolite-mediated signalling pathways. Beyond their agricultural role, such mushrooms also possess nutraceutical importance, highlighting their dual-purpose value and broad applicability in sustainable crop protection and human well-being.

Conclusion

Our study demonstrated the potential of *Lentinus squarrosulus* in suppressing tomato Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. The secretion of bioactive metabolites represents a novel and innate mechanism by which *L. squarrosulus* contributes to disease management. By influencing key defense-related pathways such as the pentose phosphate pathway, these metabolites enhance redox balance and promote the biosynthesis of protective compounds in the host. While the present work provides a foundational framework, future studies focusing on the purification and characterization of active metabolites, optimization of dosage and validation under field conditions are essential to establish *L. squarrosulus* as a sustainable biocontrol strategy in plant disease management.

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Authors' contributions

DS performed the study and wrote the manuscript. TG planned the layout and participated in drafting and editing of the manuscript. AK, SN, JSSD and MBN helped in reviewing and further drafting of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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