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Research Journal of Agricultural Sciences
Volume 14; Issue 01; pp 323–334

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Molecular Identification of Potential Antagonists and its Effect of Enzyme on Mycotoxins of *Fusarium moniliforme*

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Abstract

The *Aspergillus fumigatus, A. niger, A. ochraceus, A. oryzae, A. terreus, A. ustus, A. variecolor, Penicillium chrysogenum, P. funiculosum, Trichoderma harzianum* and *Trichoderma viride* were isolated from paddy field soil of Kanyakumari District, Tamil Nadu, India. Among the 11 fungal species were performed against bakanae disease causal agent of *Fusarium moniliforme.* According to this antagonistic activity, the *Trichoderma viride* were exhibited 72 percentage of inhibition radial growth against *Fusarium moniliforme*. The effect of culture filtrate of 5, 10, 15 and 20% of antagonistic fungi with different concentration treated against *F. moniliforme.* The 20% of *Trichoderma harzianum* were suppressed effectively than other isolated fungal species. The *T. harzianum* producing crude enzymes are amylase, cellulase, lipase, pectinase and protease were performed against *Fusarium moniliforme* by antifungal method. The protease enzyme was highly inhibited the *F. moniliforme* growth. Fungicide effect were also found against the pathogen. The hexaconazole fungicide were highly inhibited in the *F. moniliforme* growth when compared copperoxychloride fungicide. These potential fungi identified from the 18S rRNA sequence and deposited in GenBank (MW465841). The present study was determined *Trichoderma harzianum* was best biocontrol agent for bakanae disease causing soil borne pathogen *Fusarium moniliforme* in paddy field.

***Key words*:** Biocontrol, Poisoning food, Hydrolase enzymes, Antifungal, Hexaconazole, Copperoxychloride, 18S rRNA

The India is the largest rice consuming country of world. But recent decades challenges to cultivate paddy without bakanae diseases. Bakanae disease causal agent was described by Japan in 100 years ago and identified the pathogen as *Fusarium moniliforme* [1]. *Fusarium moniliforme* which causes the illness bakanae or foot rot often reduces rice yield by 10% to 50% [2]. Bakanae disease caused by *Fusarium moniliforme. Fusarium* genus is a widely distributed fungus causing many diseases in plants, animals and humans. But some of the *Fusarium* species are non-pathogenic in natural ecosystem [3]. One of the most significant fungal genera *Fusarium* that can produced mycotoxins. *Fusarium* producing mycotoxins are fumonisins, zearalenone, deoxynivalenol and other trichothecenes. Most of the *Fusarium* species are cosmopolitan and also plant pathogens. The genus of *Fusarium* containing more than 1500 species and several species are occurred in mycotoxins producer.

Bakanae disease was possible to controlled by chemical fungicides or biological treatment. An overuse of chemical fungicides over time has negatively impacted non-target populations of microorganisms, disrupting the rhizobial microbial community which results in poor soil health. Chemical fungicides which are widely employed in agro ecosystems to manage plant diseases can have a negative impact on the environment and human health. Additionally, the resistance to many chemical active components grows as fungal diseases proliferate [4]. This has happened in addition to causing the development of resistance in the target fungal strains [5]. So, the bakanae disease control management process should be way for ecofriendly [6].

The more effective and environmentally sound control measures using antagonistic microorganisms have been alternatively explored to control bakanae disease [7]. Biological control agents were reported to play a key role in the successful management of fungal plant pathogens [8]. Biological or abiotic inducers can boost a plant's immune system with or without pathogen infection [9]. A promising and secure alternative to the chemical fungicides used to control pathogens is biological

Received: 07 Nov 2022; Revised accepted: 05 Feb 2023; Published online: 18 Feb 2023

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Citation: Rajathi S, Murugesan S, Ambikapathy V, Panneerselvam A. 2023. Molecular identification of potential antagonists and its effect of enzyme on mycotoxins of *Fusarium moniliforme*. *Res. Jr. Agril Sci*. 14(1): 323-334.

control methods based on the use of common microbial populations and their secondary metabolites. These methods may also be used successfully in conjunction with chemicals [10]. Being able to effectively defend against pathogens due to their ability to colonize roots and their beneficial plant interactions that can stimulate host defense responses against pathogen infection, the use of native microorganisms to control pathogenic fungal species has acquired attention in recent years [11]. It is well known and widely employed in biocontrol strategies how crucial beneficial bacteria are to increasing nutrient availability, fostering plant development, combating soil-borne diseases and prepping the plant's immune system [12-17].

Bacterial agents like *Agrobacterium, Bacillus* and *Pseudomonas*, as well as fungal agents like *Ampelomyces, Aspergillus, Candida, Coniothyrium, Gliocadium, Pseudozyma, Streptomyces* and *Trichoderma* are among the identified biocontrol agents at the moment [18]. *Trichoderma* spp. is a genus of fungi that can be found in soil, rotting wood, plants, and the ocean. It belongs to the family Hypocreaceae. Numerous species have been shown to be opportunistic, avirulent, symbiotic and capable of acting as biological control agents against significant plant pathogenic fungi [19]. When the *Trichoderma* interact with plants to induced the gene expressions for defense mechanism against pathogen [20-21].

The *Trichoderma* spp. have the power to promote plant development while inhibiting the growth of fungal diseases. However, the bio-efficacy of *Trichoderma* spp. in promoting plant growth and controlling the bakanae disease particularly in the rice variety MR 219 [22]. The antagonistic properties of *Trichoderma* species based upon the direct and indirect mechanisms. Direct mechanisms based mycoparasitisms and production of enzymes with metabolites. An indirect mechanism can be act as synergistically [23]. In the present investigation we evaluated about the potentials of antagonistic activity against *Fusarium moniliforme* by *Trichoderma harzianum* with culture filtrates. The potential antagonists producing enzymes are estimated and also biocontrol measurements were compared with the chemical treatment of fungicides against bakanae disease causing soil borne pathogen *Fusarium moniliforme*. The potential antagonist was confirmed the genus and species by 18S rRNA gene sequence.

MATERIALS AND METHODS

Isolation of fungi [24]

Potato Dextrose Agar (PDA) plates with streptomycin (50mg/mL1) were utilized. In Kanyakumari, Tamil Nadu, India, soil samples from paddy field were used to isolate the fungal strains. The soil suspension from the serial dilution were prepared with sterile water. A random selection of fungal colonies were moved to a new medium for purification after 2–5 days of incubation.

Identification of fungal colonies [25]

The fungal colonies were characterized by colour and growth morphology on the growth medium. The spores structures and arrangements were identified by using electron microscope. After the 7 days of incubation, fungi grown on the PDA petri plate was transferred to PDA slant.

Dual culture method [26]

Potential; antagonist's culture blocks of 5mm diameter cut from the margin of 8 days old cultures of both test pathogen and antagonists were placed opposite to each other on PDA in 90mm glass Petri plates. The inoculated plates were incubated at room temperature (28±2°C). The colony growth and radial growth of the pathogen was measured at both sides i.e., towards and opposing each other from their central loci. Intermingled inhibition zone was also measured at the same period. The assessment of interaction was made followed the model of Skidmore and Dickinson, 1976. The parameters used for the assessment of colony interaction were the width of inhibition zone of growth intermingled zone and percentage inhibition of radial growth, i. e. $100 \times (r_1 - r_2)/ r_1$, where, r_1 denotes the radial growth of the paddy pathogen towards the opposite side and r_2 denotes the radius of the pathogen towards the antagonist to fungi.

Culture filtrate method [27]

The effect of culture filtrate, the antagonist fungi were grown on PDA medium. Three mycelia agar discs (each of 5mm diameter) of an individual antagonist, were cut from the actively growing margins of 5-day old culture and were inoculated into a 250ml conical flask containing 100ml Potato Dextrose broth medium. After 10 days of incubation at (30 ± 2°C) the culture of an antagonist was filtered through a filter paper and then centrifuged at 3000rpm for 20 minutes and finally filter through a micro pore filter paper under vacuum pressure to obtain the cell free culture filtrate. The Culture filtrate of an antagonist containing its non – volatile metabolites was tested in three concentrations (5, 10, 15 and 20%) against *Fusarium moniliforme*. The culture filtrate of a particular concentration was obtained by supplemented it with required amount of sterilized PDA medium. Each Petri plate was inoculated centrally with a 5mm. mycelia agar disc cut from the margin of actively growing culture of a *Fusarium moniliforme* pathogen. All the plates were incubated at room temperature (28 ± 2°C). The radial growth of the colonies was measured after five days of incubation. The percentage growth inhibition of a rice pathogen was calculated by using the formula:

$$I = C - \frac{T}{C} \times 100$$

Where;
I- Percent inhibition of the pathogen
C- Radial growth of the pathogen in control
T- Radial growth of the pathogen in treatment

Effect of fungicides on Fusarium moniliforme [28]

Two fungicides were used for fungicide on the *Fusarium moniliforme*. Dilutions of the fungicides were prepared by mixing appropriate amounts of each fungicide in sterile distilled water and stored at 4°C until used. A 2% (w/v) milled wheat agar was used. Media were autoclaved at 120°C for 20 min before cooling to 50°C at the time of fungicides were added to obtain the required concentration (100, 200, 300, 400 and 500ppm). Media with fungicide were added and control treatments per each W. Flasks of molten media were thoroughly shaken, prior to pouring into sterile Petri dishes.

Enzyme assay
Amylase [29]

Amylase activity was assayed by growing the fungi on starch medium (starch - 2 g, peptone - 1 g, yeast extract - 1 g, agar - 20 g; distilled water-lL. After 5-10 days, the plates were flooded with 1% aqueous IKI solution. A yellow zone around the colony in an otherwise blue medium was considered as positive test for starch hydrolysis.

Cellulolytic enzymes [29]

Three methods were used to detect cellulolytic enzymes (cellulase). The cellulolysis basal medium (CBA) consisted of:

C4H12N206 - 5 g, KH2P04 - 1 g, MgS04.7H20 - 0.5 g, yeast-extract - 0.1g, CaCl2.2H20 - 0.001 g, distilled water - 1L.

Cellulolysis Basal Medium supplemented with 1.8% w/v agar was transferred in 10mL aliquots to glass culture bottles, autoclaved, and allowed to solidify. Then gently and carefully mixed viscous 0.1mL CBM medium supplemented with 1% w/v cellulose azure (azure I dye, C.I.5201O) and 1.8% w/v agar was added to the surface of the solidified agar as an overlay. The BI-layered medium was inoculated with discs of the test fungi and incubated. The migration of dye into the clear lower layer indicated the presence of cellulase.

Lipase [30]

Lipase activity was determined by growing the isolates on a medium containing a lipid (Tween 20, Sigma Chemical Co.) as the primary source of carbon. The medium was as follows: peptone - 1 g, yeast extract - 0.1 g, agar 18g, Tween 20 - 10 mL (autoclaved separately from the rest of the medium), distilled water - 990 mL. A positive test was the occurrence of precipitated fatty acid crystals around the colony.

Pectolytic enzymes [31]

The medium contained 500mL of mineral salt solution, 1g yeast extract, 15 g of agar, 5g of pectin, and 500 mL of distilled water. The mineral salts solution contained per liter: 2 (NH4)2 SO_4, 4 g KH_2 PO_4, 6 g Na_2 HPO_4, 0.2 g $FeSO_4$.7 H_2O, 1 mg $CaCl_2$, 10 µg H_3BO_3, 10 µg Mn SO_4, 10 µg $ZnSO_4$, 50 µg $CuSO_4$, 10 µg*;* MoO_3, pH-7 or pH-5 as needed. This medium at pH-7 was used to detect pectate lyase production. The same medium at pH-5 was used to detect polygalacturonase activity. For all tests, plates were incubated for 5-10 days and then flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide. This reagent precipitates intact pectin in the medium and thus a clear zone around the colony in opaque medium indicated the degradation of the pectin.

Proteolytic enzymes [32]

Potential strains were grown on Casein Agar Medium (Peptic digest of animal tissue 5.00g, Beef extract 1.50g, Yeast extract 1.50g, Sodium chloride 5.00g, Agar 15.00g, Casein 10.00g and Distilled water 1000ml). The medium was aseptically transferred to petri dishes and inoculated with a 6mm agar disc cut from 5-day old fungal culture of each strain separately and incubated at 26 ± 2°C in darkness for 3 to 5 days. The plates were flooded with Bromo Cresol Green dye. The clear distinct zone indicated proteolytic activity. A distinct zone surrounded by greenish-blue colour is pH dependent (8.0±0.2). The proteolytic activity appeared as a colourless zone, while the rest of the plates as greenish-blue in colour.

18S rRNA sequence [33]

Fungal strain was amplified from each 18S rDNA by PCR using the universal fungal primers. The optimal conditions for PCR amplification of 18S rDNA segments with these primers. The purified PCR products were used in sequencing reactions with the same set of primers using a BigDye Terminator Cycle Sequencing Ready Reaction Kit version 3.0 (Applied Biosystems, Foster City, Calif.). Sequencing was performed on an ABI 3100 sequencer (Applied Biosystems). The fungi were sequenced from both directions. The obtained sequences have been submitted to GenBank.

Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

RESULTS AND DISCUSSION

Isolation of fungi from paddy field soil sample Kanyakumari

According to isolation of fungal colonies such as *Aspergillus fumigatus, A. niger, A. ochraceus, A. terreus, Cephalosporium* sp., *Cladosporium* sp., *Cunninghamella verticillata, Fusarium oxysporum, Memnoniella* sp., *Penicillium granulatum, Penicillium* sp. and *Trichoderma harzianum*were isolated from paddy field soil recorded respectively (Plate 1).

Efficacy of biocontrol effect against Fusarium moniliforme

Twelve fungal colonies were performed as biocontrol agent against pathogenic fungi of *Fusarium moniliforme* through the dual culture experiments. All the fungal cultures were inhibited in the pathogen (Plate 2).

Colony growth of the antagonistic fungi towards pathogen

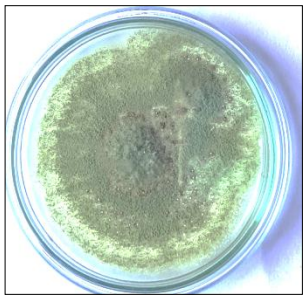
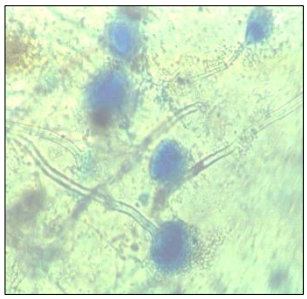
Aspergillus fumigatus, A. niger, A. ochraceus, A. terreus, Cephlosporium sp., *Cladosporium* sp., *Cunninghamella verticillata, Fusarium oxysporum, Memnoniella*sp., *Penicillium granulatum, Penicillium* sp. and *Trichoderma harzianum* exhibited following growth such as10.0±2.10, 4.01±1.03, 5.01±2.02, 9.05±1.01, 9.06±2.17, 1.05±0.97, 3.11±0.98, 2.00±0.99, 1.02±0.65, 4.05±1.12, 4.11±1.17 and 5.01±2.11mm towards *Fusarium moniliforme* (Table 1).

Table 1 Effect of antagonistic fungi on the growth of inhibition of *F.moniliforme* by *invitro* method

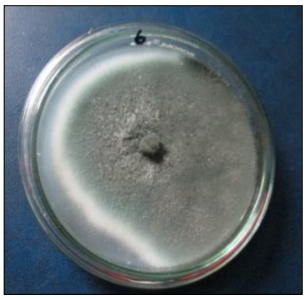
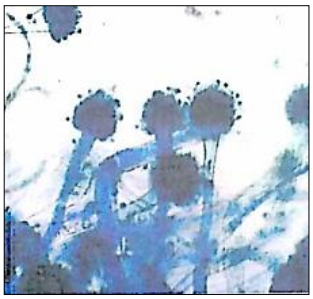
Name of the fungi	Zone of inhibition (mm)			
	A	B	C	D
Aspergillus fumigatus	10.0±2.10	12.3±4.01	9.05±0.07	11.0±3.66
A.niger	4.01±1.03	11.3±3.76	8.04±0.04	4.66±1.55
A.ochraceus	5.01±2.02	9.33±3.11	10.6±0.06	6.00±2.33
A. terreus	9.05±1.01	15.6±5.02	6.03±0.06	10.1±1.33
Cephalosporium sp.	9.06±2.17	12.0±4.00	7.05±0.03	11.6±3.86
Cladosporium sp.	1.05±0.97	15.0±5.01	11.5±0.09	2.01±1.07
Cunninghamella verticillata	3.11±0.98	12.3±4.01	10.2±0.10	3.91±1.97
Fusarium oxysporum	2.00±0.99	11.3±3.76	11.8±0.08	2.08±1.50
Memnoniella sp.	1.02±0.65	17.0±5.66	7.02±0.08	1.97±0.91
Penicillium granulatum	4.05±1.12	12.1±4.04	11.6±0.12	5.97±2.97
Penicillium sp.	4.11±1.17	16.0±5.33	8.06±0.05	5.02±2.08
Trichoderma harzianum	5.01±2.11	19.0±6.33	12.5±0.10	6.10±3.17

Standard deviation ± Standard error

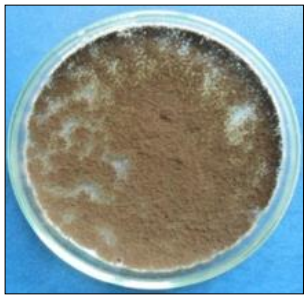
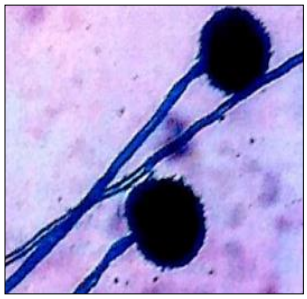
A-Colony growth of the antagonistic fungi towards pathogen, **B**-Colony growth of the antagonistic fungi away from the pathogen, **C**-Colony growth of the pathogen towards the antagonistic fungi and **D**-Colony growth of the pathogen away from the antagonistic fungi

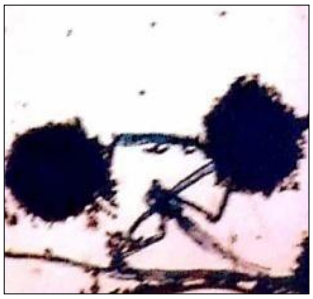
Aspergillus flavus

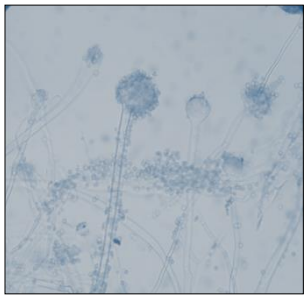
A. fumigatus

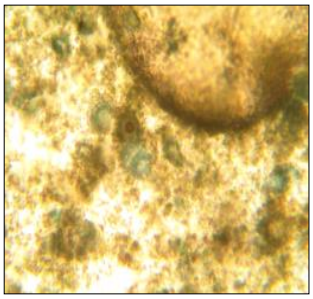
A. niger

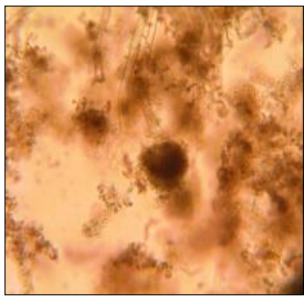
A. ochraceus

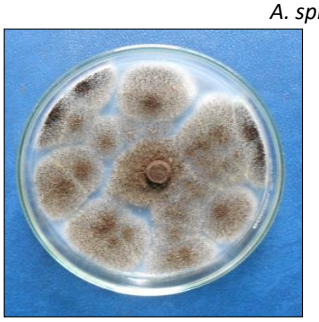
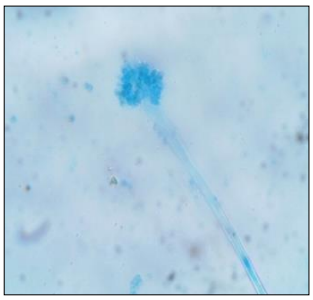
A. oryzae

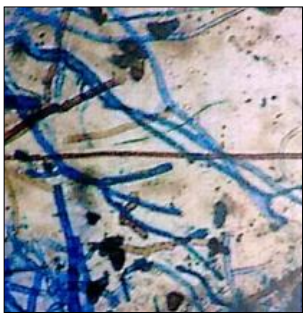
A. spinulosus

A. terreus

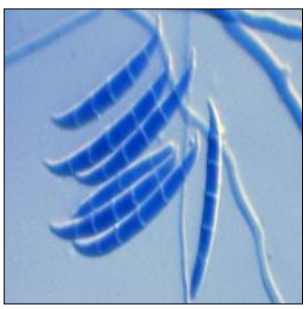



A. variecolor

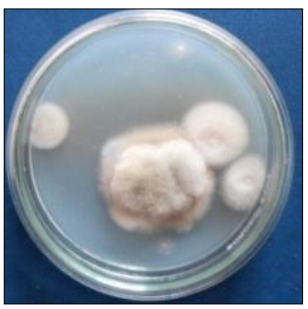



Curvularia lunata

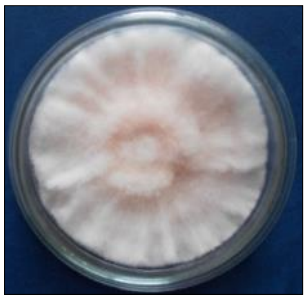
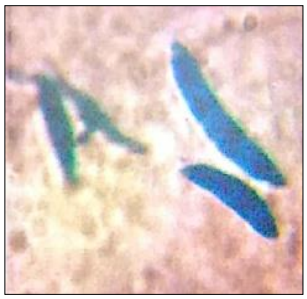

Curvularia sp.,

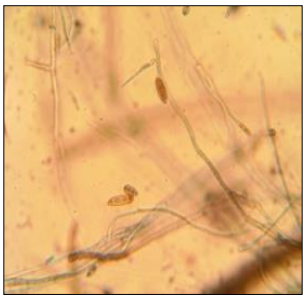



Fusarium moniliforme

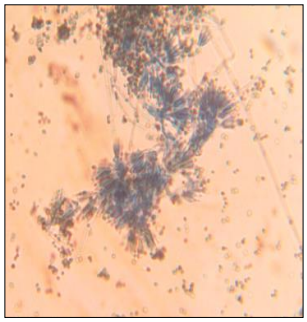


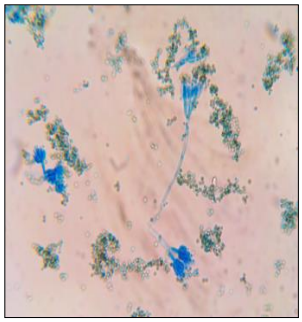

Fusarium oxysporum

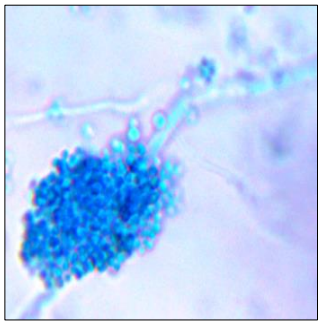
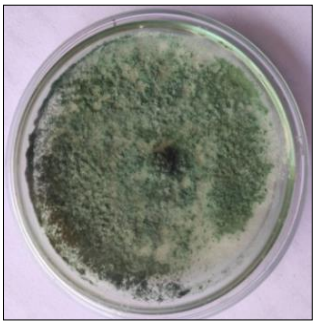
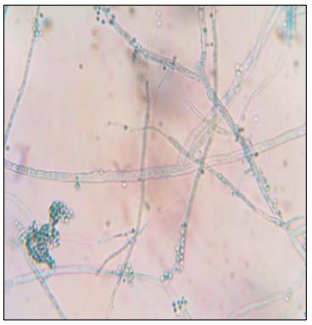


Fusarium solani *Helminthosporium oryzae*

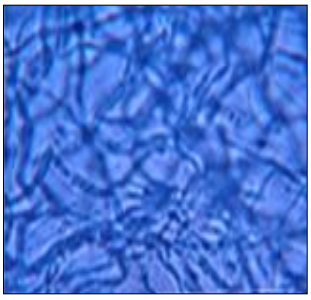





Penicillium citrinum *P. chrysogenum*

Trichoderma viride *T. harzianum*

Verticillium sp.

Plate 1 Pure culture of fungal species isolated from the soil of paddy field

Colony growth of the antagonistic fungi against the pathogen

The fungal colonies of *Aspergillus fumigatus, A. niger, A. ochraceus, A. terreus, Cephlosporium* sp., *Cladosporium* sp., *Cunninghamella verticillata, Fusarium oxysporum, Memnoniella*sp., *Penicillium granulatum, Penicillium* sp. and *Trichoderma harzianum* such as 12.3±4.01, 11.3±3.76, 9.33±3.11, 15.6±5.02, 12.0±4.00, 15.0±5.01, 12.3±4.01, 11.3±3.76, 17.0±5.66, 12.1±4.04, 16.0±5.33 and 19.0±6.33mm was away from the *Fusarium moniliforme* (Table 1).

Colony growth of the pathogen towards the antagonistic fungi

The pathogenic fungi of *Fusarium moniliforme* was 9.05±0.07, 8.04±0.04, 10.6±0.06, 6.03±0.06, 7.05±0.03, 11.5±0.09, 10.2±0.10, 11.8±0.08, 7.02±0.08, 11.6±0.12, 8.06±0.05 and 12.5±0.10mm grown towards *Aspergillus fumigatus, A. niger, A. ochraceus, A. terreus, Cephlosporium* sp., *Cladosporium* sp., *Cunninghamella verticillata, Fusarium oxysporum, Memnoniella*sp., *Penicillium granulatum, Penicillium* sp. and *Trichoderma harzianum* recorded respectively (Table 1).

Colony growth of the pathogen away from the antagonistic fungi

Among the 12 fungal colonies were observed in the dual culture experiment and the colony growth of the pathogen away from the antagonistic fungi. *F. moniliforme* growth is 11.0±3.66, 4.66±1.55, 6.00±2.33, 10.1±1.33, 11.6±3.86, 2.01±1.07, 3.91±1.97, 2.08±1.50, 1.97±0.91, 5.97±2.97, 5.02±2.08 and 6.10±3.17mm away from the respective fungal colonies are *Aspergillus fumigatus, A. niger, A. ochraceus, A. terreus, Cephlosporium* sp., *Cladosporium* sp., *Cunninghamella verticillata, Fusarium oxysporum, Memnoniella*sp., *Penicillium granulatum, Penicillium* sp. and *Trichoderma harzianum* (Table 1).

Table 2 Effect of culture filtrates of antagonistic fungi against *F. moniliforme*

Name of the fungi	Growth measurement (mm)			
	5%	10%	15%	20%
Aspergillus fumigatus	2.25±1.15	0.00±0.00	0.00±0.00	0.00±0.00
A. terreus	3.15±2.09	2.10±1.03	1.07±0.98	0.00±0.00
A. niger	2.05±1.01	0.09±0.00	0.00±0.00	0.00±0.00
Cephalosporium sp.	2.20±1.10	1.15±1.09	0.10±0.03	0.00±0.00
Memnoniella sp.	3.05±2.01	1.20±1.10	0.95±0.50	0.00±0.00
Penicillium sp.	2.13±1.06	1.05±0.98	0.00±0.00	0.00±0.00
Trichoderma harzianum	2.10±1.03	0.00±0.00	0.00±0.00	0.00±0.00

Standard deviation ± Standard error

Table 3 Different concentration of food poisoning effect of *F. moniliforme*

Name of the fungicides	Concentration (ppm)	Growth of inhibition (mm)
Hexaconazole	100	0.11±0.07
	200	0.92±0.05
	300	0.75±0.03
	400	0.53±0.01
	500	0.00±0.00
Copperoxychloride	100	0.10±0.04
	200	0.96±0.03
	300	0.71±0.01
	400	0.00±0.00
	500	0.00±0.00

Standard deviation ± Standard error

Effect of culture filtrates of antagonistic fungi on Fusarium moniliforme

All the fungal colonies metabolites were inhibition on mycelial growth of*Fusarium moniliforme*at different concentration of 5, 10, 15 and 20% of antagonitic fungi. The crude metabolites of *Aspergillus fumigatus*and *Trichoderma harzianum* were 100% percent inhibited *F. moniliforme* growth at 10, 15 and 20% of culture filtrate. The *A. niger* and *Penicillium* sp. were completely inhibited the pathogen at 15 and 20% of filtrate. The *A. terreus*, *Cephalosporium* sp. and *Memnoiella* sp. were inhibited the pathogen at 20% of culture filtrate were observed (Table 2, Plate 3).

Effect of fungicides against F. moniliforme

The various concentration of different fungicides contain media were inoculated on the *F. moniliforme* growth stages were observed. The hexaconazole treated media was 0.11±0.07mm, 0.92±0.05mm, 0.75±0.03mm and 0.53±0.01mm growth inhibition observed from the 100, 200, 300 and 400ppm on the pathogen respectively. The hexaconazole 500ppm completely inhibited the *F. moniliforme* growth. The copperoxychloride treatment of 100ppm was 0.10±0.04mm growth of *F. moniliforme*, 200ppm has 0.96±0.03mm growth, 300ppm exhibited 0.71±0.01mm, 400 and 500ppm inhibited pathogen found to be recorded against *F. moniliforme* were recorded (Table 3, Plate 4).

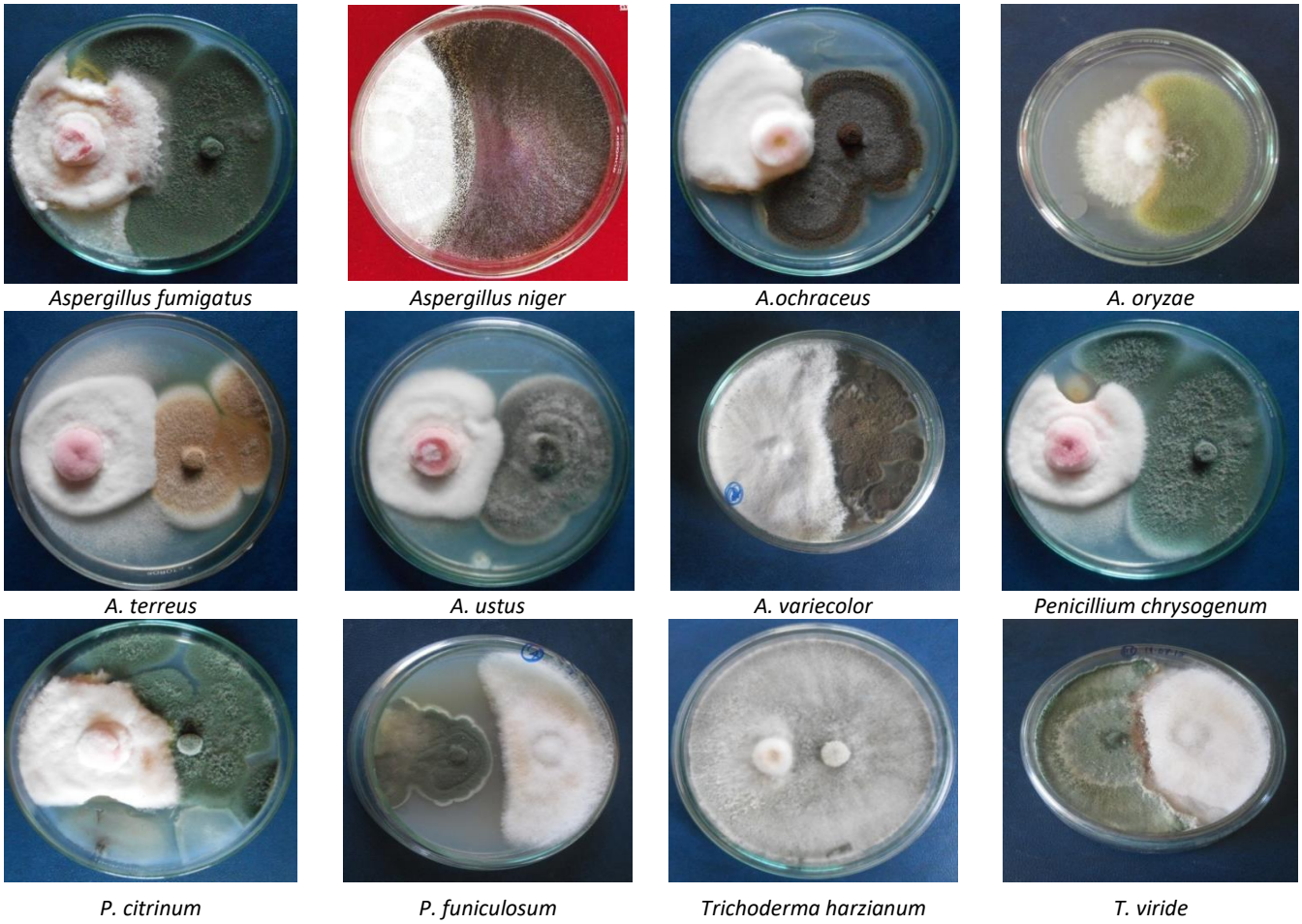


Plate 2 Effect of antagonistic fungi on the growth of *F. moniliforme* by *invitro* method

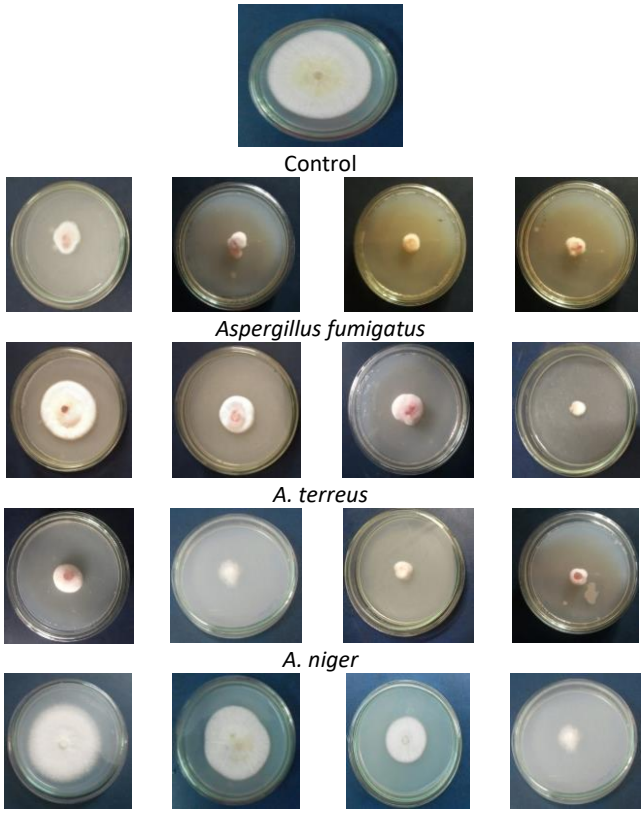


Plate 3: Effect of culture filtrate of antagonistic fungi against *F.moniliforme*

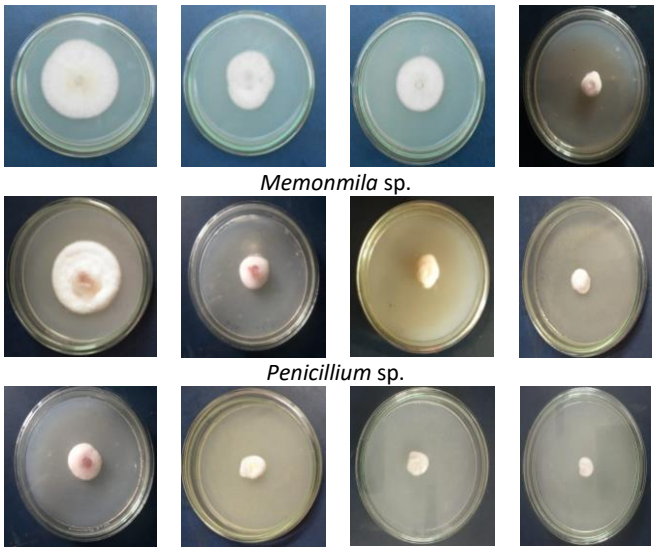


Plate 4 Different concentration of food poisoning effect of *F. moniliforme*

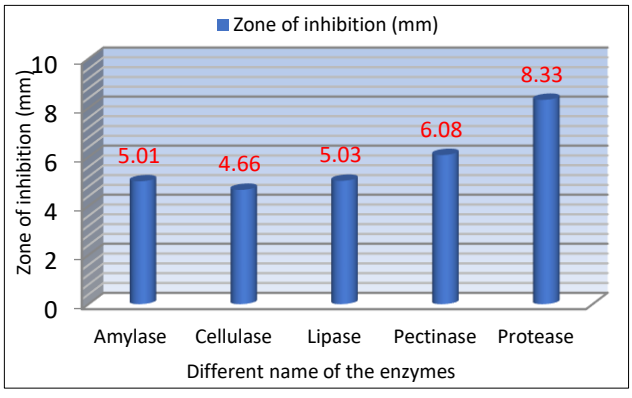


Fig 1 Effect of potential antagonistic fungal enzymes against *F. moniliforme*

Effect of potential antagonistic fungi enzymes against F. moniliforme

The enzymes amylase, cellulase, lipase, pectinase and protease were purified from *Trichoderma viride* by using *in-vitro* methods. The enzymes 100µl were used for antifungal maximum inhibitionactivity against the *Fusarium moniliforme* pathogen. Amylase, cellulase, lipase, pectinase and protease enzymes are inhibited the *Fusarium moniliforme* inthe zone of inhibition such as 5.00±1.66, 4.66±1.55, 5.00±0.84, 6.00±2.00 and 8.33±2.77mm was recorded respectively (Fig 1, Plate 5-6).

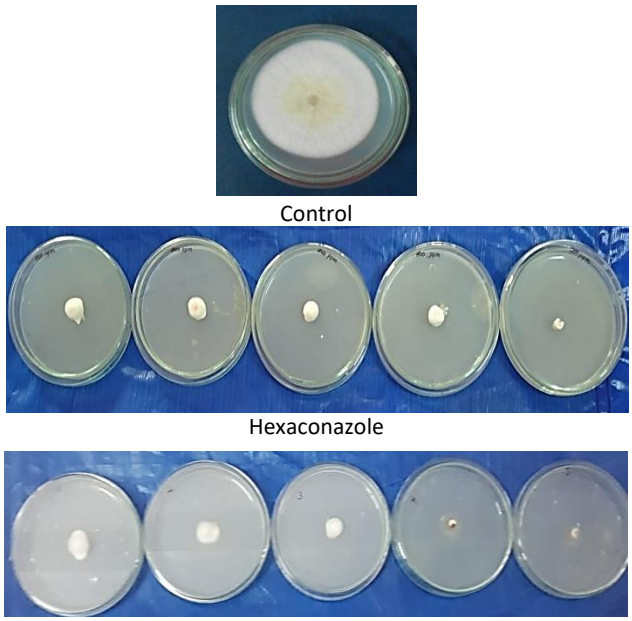
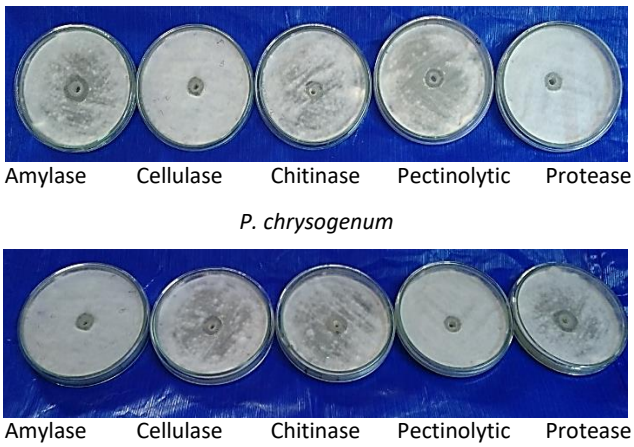
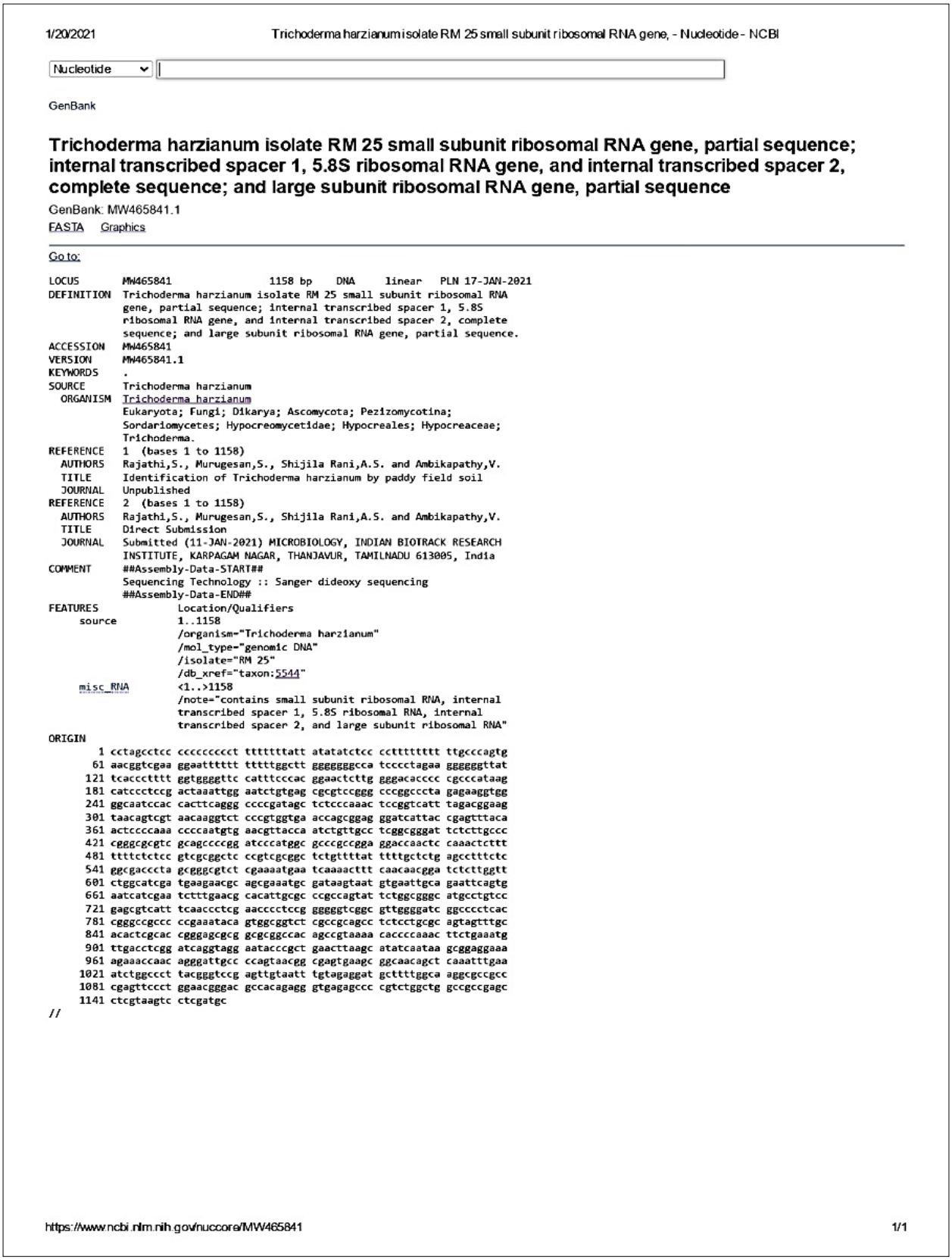


Plate 5 Estimation of enzymes from *T. harzianum*

Amylase Cellulase Chitinase Pectinolytic Protease

Plate 6 Effect of potential antagonistic fungal enzymes against *F. moniliforme*





1/20/2021 Trichoderma harzianum isolate RM 25 small subunit ribosomal RNA gene, - Nucleotide - NCBI

Nucleotide

GenBank

Trichoderma harzianum isolate RM 25 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MW465841.1

FASTA Graphics

Go to:

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VERSION     MW465841.1
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            Trichoderma.
REFERENCE   1  (bases 1 to 1158)
  AUTHORS   Rajathi,S., Murugesan,S., Shijila Rani,A.S. and Ambikapathy,V.
  TITLE     Identification of Trichoderma harzianum by paddy field soil
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1158)
  AUTHORS   Rajathi,S., Murugesan,S., Shijila Rani,A.S. and Ambikapathy,V.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-JAN-2021) MICROBIOLOGY, INDIAN BIOTRACK RESEARCH
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https://www.ncbi.nlm.nih.gov/nuccore/MW465841 1/1

Fig 2 Molecular identification of *Trichoderma harzianum*

Potential fungi was confirmed by 18S rRNA gene sequencing

The micro fungi was identified as *Trichoderrma harzianum* and this was deposited in NCBI database with gene bank accession number MW465841 (Fig 2).

In recent study of *Trichoderma harzianum* isolate was evaluated the antagonism against seedborne fungal pathogens of *Alternaria alternata, Bipolaris cynodontis, Fusarium culmorum* and *F. oxysporum*. The antagonist fungi were highly controlled *F. culmorum* pathogen mycelia growth [34]. The phytopathogen of *Fusarium moniliforme* was controlled by endophytic bacteria isolated from sugarcane root and stem. Totally, 52 fungal colonies were isolated, among the fungi only 14 species were effectively performed against pathogen. The endophytic bacteria were determined such as *Asaia bogorensis, Burkholderia gladioli, Tanticharoenia aidae, Nguyenibacter vanlangensis, Acidomonas methanolica* and *Bacillus altitudinis.* The *B. gladioli* was 80% controlled the pathogen [35]. The novel pathogen of *Fusarium sudanense* was controlled by *Trichoderma harzianum* with different hydrophilic water activity and temperature such as 15°C and

25°C. The *T. harzianum* was suppressed *F. sudanense.* This inhibition was increased in sixth day incubation [36]. *Trichoderma harzianum* and *Trichoderma viride* strain have been reported to possess antagonistic activity against *Fusarium oxysporum* strain, demonstrating mycelial growth inhibition rates of 75.7% and 67.7% found to be recognized respectively [37]. The *T. harzianum* strain's antagonistic efficacy against *F. oxysporum*, with a mycelial inhibition rate of 45.69% [38]. The *Trichoderma harzianum* was used for the biological control measurement of *Fusarium moniliforme.* The *F. moniliforme* was isolated from the rotted *Dioscorea rotundata* tubers and also *Botryodiploidia theobromae, Aspergillus flavus, A. niger, A.ochraceus, F. oxysporum, Penicillium purpurogenum* and *Petalotia* sp. were found in the sample. The *T. harzianum* has significant inhibition rate of 58.70% an effect on the *F. moniliforme* [39]. An eight *Trichoderma* strains were examined their antagonistic effect against *Fusarium graminearum* by dual culture experiments. The *Trichoderma* strains were inhibited the pathogen of *F. graminearum* mycelial growth. An especially found the over growth of *T. longibranchiatum, T. harzianum, T. atroviride* and *T. asperellum* sporulated on the colony of *F. graminearum* [40]. The interactions between eight isolates of *Trichoderma* species and *Fusarium moniliforme*. The two isolates of *T. harzianum* T_{23} and T_{16} had significant antagonistic potential, according to the findings of dual culture and tests on volatile activity [41]. *Bacillus subtilis* and *Trichoderma harzianum* was performed against the root rot disease causing *Phytopthora capsici* and *Rhizoctonia solani* in pepper plant under the greenhouse environment. In addition of *T. harzianum* was effectively controlled *R. solani* but not significant reduction against *P. capsici* [42]. The soil fungi of *Aspergillus candidus, A. niger, A. sydowi, A. sulphureus, Gliocladium* sp., *Penicillium citrinum, Trichoderma harzianum* and *T. viride* were performed against the *Fusarium solani* by *invitro*antagonistic activity. They have reveals maximum percentage of pathogen growth inhibition by *A. niger*followed by *T. viride, Gliocladium* sp., *T. harzianum* and *P. citrinum* [43]. *Fusarium moniliforme* were controlled by endophytic microorganisms of *Bacillus subtilis* and *Trichoderma* sp. effectively. These two microorganisms were inactivated the fumonisms mycotoxins from the *F.moniliforme* [44]. *Trichoderma virens* and *Trichoderma harzianum's* antagonistic hyphae encircling and penetrating the hyphae of *Fusarium solani* and lysing them [45-46]. Most of the antagonistic studies were performed with universal biocontrol agent of *Trichoderma* species especially the *Trichoderma harzianum* was most effective biocontrol agent for plant pathogens. In this present investigation of an effect of antagonistic fungi on the growth of inhibition of *F.moniliforme* by *invitro* method was performed with all the paddy field soil isolates of Kanyakumari such as *Aspergillus fumigatus, A. niger, A. ochraceus, A. terreus, Cephalosporium* sp., *Cladoporium* sp., *Cunninghamella verticillata, Fusarium oxysporum, Memnoniella* sp., *Penicillium granulatum, Penicillium* sp. and *Trichoderma harzianum.* Among the soil fungi, the *Trichoderma harzianum* was highly inhibited the pathogen of *F. moniliforme* growth.

The *Trichoderma viride* culture filtrates inhibited *Fusarium proliferatum* and *Fusarium verticillioides* mycelial growth at rates of 32.3% and 56.7%, respectively, while *Trichoderma harzianum* recorded mycelial inhibition rates of 23.5% and 44.09% [47]. The poison food technique, the effectiveness of 15-day-old culture filtrate (nonvolatile chemical) of 20 *Trichoderma* isolates was assessed against *Colletotrichum graminicola* mycelial growth at 10% and 25% concentration. At 10% and 25% concentration, all 19 isolates of *Trichoderma asperellum* and one isolate of *Trichoderma harzianum* significantly inhibited the growth of *C. graminicola* mycelia in comparison to the control. The greatest *C. graminicola* mycelia growth suppression was seen at 10 and 25% of the T4 isolate, resulting in respective radial growth inhibition values of 68.18% and 80.68%were observed [48]. The effectiveness of *Trichoderma* isolate culture filtrates against strains of *F. oxysporum* and they attributed this effectiveness to the formation of active secondary metabolites [49]. The hundred strains of *Trichoderma* sp. were performed against *Fusarium graminearum* by *in-vitro* antagonistic activity. Among the 100 strains the *T. harzianum* CCTCC-RW0024 strain was highly inhibited the pathogen growth (96.3%) and also this strain produced secondary metabolites was significantly decreased (54.54%) the pathogen growth in 100µg concentration [50]. The mycelial inhibition rates of culture filtrates of *T. viride* and *T. harzia*num strains against a strain of *F. oxysporum* at a concentration of 5% v/v and reported mycelial inhibition rates of 51.53% and 24.71%, respectively [51]. Brinjal and tomato wilt disease causing *F. solani* and *F. oxysporum* were performed food poisoned technique with *T. harzianum, T. atroviride* and *T. longibrachiatum* of 25, 50 and 75% by *in-vitro* method. The concentration of 50 and 75% of *T. harzianum* showed maximum inhibition of *F.oxysporum* mycelia growth [52]. *Phalaris minor* L. and *Rumex dentatus* L., two troublesome weeds of wheat were used as test subjects for the herbicidal potential of culture filtrates from four *Trichoderma* species: *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai, *Trichoderma reesei* Simmons, and *Trichoderma viride* Pers. Generally, all four *Trichoderma* species' compounds greatly decreased the two target weed species' varied root and shoot growth indices. With the exception of *T. harzianum*, the original concentrations of the culture filtrates of all *Trichoderma* spp. considerably decreased a number of parameters related to the root and shoot growth of wheat seedlings [53]. In the present investigation of poison food technique showed the pathogen growth reduction caused by maximum with *Trichoderma harzianum* and *Aspergillus flavus* at 10, 15 and 20% of culture filtrate. The *T. harzianum* was highly inhibited the pathogen than the *A. fumigatus* at 5% of culture filtrate.

The different concentrations of aureofungin, streptocycline, copper oxychloride and carbendazim were performed against the Pokkah boeng causing *Fusarium moniliforme* by food poisoning technique. Among the fungicides, the Copper oxychloride was found more effective inhibition at 250ppm against pathogen of *F. moniliforme* [54]. The fungus mycelial development was constrained proportionately to the rise in fungicidal concentration, with the largest reduction observed at concentrations of 300ppm and 200ppm. Similar to this, fungicide Carbendazim 12% + Mancozeb 63% WP was found to have the highest fungitoxic effect followed by Captan 70% + Hexaconazole 5% WP and Carbendazim 50% WP with no statistically significant difference at any concentration while fungicide Mancozeb 75% WP had the least effect on the fungus' ability to grow mycelia [55]. The broad-spectrum fungicides known as methyl benzimidazole carbamate (MBC) fungicides, of which carbendazim is a member, are crucial in the fight against fungal diseases that affect numerous agricultural crops [56]. Resistance of fusarial strains to methyl benzimidazole carbamate fungicides was reported in previous studies [57]. When methyl benzimidazole carbamate fungicides bind to b-tubulin, tubulin production is inhibited and fungal cell mitosis is suppressed [58]. In this current study were revealed the fungicidal culture filtrate had been performed with hexaconazole and copper oxychloride. The 400 and 500ppm

copper oxychloride was showed maximum reduction of pathogen growth by *invitro* poison food technique.

At a 25% culture-free filtrate concentration in *in-vitro* experiments, all the chitinase producing *Trichoderma* isolates reduced the growth of the pathogens *Dematophora necatrix, Fusarium solani, F. oxysporum* and *Pythium aphanidermatum*. Additionally, isolate *Trichoderma harzianum* showed the best disease control and lowest wilt incidence *on F.oxysporum* under *in-vivo* circumstances, with mean wilt incidence and disease control of 21% and 48%, respectively [59]. The impact of culture filtrate on the growth of some well-known phytopathogens, including *D. necatrix, F. oxysporum, F. solani* and *P. aphanidermatum* revealed that isolated *Trichoderma* strains producing chitinase significantly inhibited the growth of all the tested pathogens. Concentrations and isolates also differed significantly from one another. There have been reports of *Trichoderma* culture free filtrate inhibiting pathogen growth [60-61]. It was discovered that culture filtrates of *T. harzianum* limit zoospore germination, germ tube elongation, and mycelial growth of *P. aphanidermatum* producing damping-off disease of tobacco. This impact of *Trichoderma* culture filtrate on the pathogen may be due to the generation of chitinase [62]. Obviously, *Trichoderma* sp. produced chitinase at levels as low as 0.061 U/mL with a great deal of variety among the isolates [63]. The *Trichoderma* strains antagonistic activity is typically correlated with the activity of hydrolytic enzymes particularly chitinase (chitin) and (1,3) glucanase (pachyman). Antagonism between *Trichoderma* strains may be connected to the extracellular enzymes they produce. *Trichoderma* produces lytic enzymes like chitinases that directly destroy the plant pathogen [64]. These enzymes have a tight connection to mycoparasitism but they are also assisting in the development of host-induced systemic resistance (ISR) to pathogen infection [65-67]. In this study regards the effect of potential antagonistic (*T. harzianum*) fungal hydrolysate enzymes are amylase cellulase, lipase, pectinase and protease were performed at 100µl concentration for antifungal activity against the pathogen. These enzymes were attributed to the antagonistic activity. The protease enzyme was highly inhibited the pathogen growth when compared with other enzymes such as pectinase, lipase, amylase and cellulase.

CONCLUSION

In the current scenario challenge is prevent the bakanae disease of *Oryza sativa.* There is available many fungicides to control the pathogen of bakanae disease as *Fusarium moniliforme.* Especially the Kanyakumari district was mostly affected the paddy crops by *F. moniliforme.* They have to controlled by chemical fungicides implementation. But the soil has loss the fertility, microflora, minerals and nutrients. Therefore, this study aimed to controlled the respective disease by the mode of ecofriendly and biologically approach in sustainable able control of pathogen. So, the native mycoflora was isolated from the paddy field soil, Kanyakumari. Unexpectedly all isolates have the capability of antagonistic effect against the *F. moniliforme.* Especially the *Trichoderma harzianum* was most effective than other isolates for pathogen control measurement. The pathogen producing mycotoxins was reduced by the *T. harzianum* produced metabolites and its hydrolysate enzymes. According to this investigation I would like to suggest the control measurement of bakanae disease in *O. sativa* crops only applying the *T. harzianum* secondary metabolites. Because these secondary metabolites were not harmful for the agricultural field fertility as well as human and animal health.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

Dr. S. Murugesan was designed and finalized the manuscript of research, Mrs. S. Rajathi was collected samples and analyzed her research work in superficially work and prepared the draft manuscript. Dr. S. Murugesan, Dr. V. Ambikapathy and Dr. A. Panneerselvam were read and approve the final version of the manuscript.

Acknowledgements

The authors are grateful to the Head, Department of Botany, Periyar University, Salem, for providing laboratory facilities during the period of investigation.

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