Mycofungicide To Control the Collar-Rot Pathogen of *Mentha Arvensis* L.

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Summary

Mentha arvensis (family Labiatae) is an important essential oil yielding herb growing throughout the world. The collar rot disease caused by soil borne pathogen *Sclerotium rolfsii* Sacc. damages the crop. The use of fungicides is not allowed as the oil is used in pharmaceutical. Usage of bioagent as an alternative method to control collar- rot is recommended. The bio-control agent like *Trichoderma* has gained importance because of its potentiality in controlling fungal phytopathogens. Hence, a study was conducted to isolate and to assess the antagonist effect of *Trichoderma* isolates against *S. rolfsii*. A total of forty-five isolates of *Trichoderma* were obtained from soil samples of mentha growing field. These isolates were tested invitro against *S. rolfsii*. Thirteen isolates were found to be very effective in controlling the growth and sclerotial formation the pathogen. Among these *T. fertile, T. gamsii, T. fasciculatum, T. neokoningii* are most effective antagonisms than other isolates of *Trichoderma*. The operative mechanism for the antagonistic effect of *Trichoderma* isolates is also studied.

Introduction

Mentha arvensis (family Labiatae) is an aromatic perennial herb, and is commercially cultivated in tropical and subtropical climates. The oil and by-product (menthol and dementholized oil) of this plant have the highest share in the global mint trades. Commercial cultivation is done to obtain its oil, which has different chemical constituents of economic importance, viz. menthol, methyl acetate, terpenes, etc. These constituents are used in medicinal preparation, toothpaste, mouthwash, perfumery, cosmetics and as flavoring agents. The menthol mint crop is extensively cultivated in India and about 70% of the international annual requirement is met from crops raised in the central region of the Indo-Gangetic plains [1].

It is reported that in India, the crop is severely affected by collar-rot disease. The disease is caused by *Sclerotium rolfsii Sacc* (Saccardo), a soil borne plant pathogen that causes considerable damage to the crop. Though collar rot and wilt disease of Mentha were reported way back in 1933 from Japan [2], no further studies were undertaken on this disease. The first attempt to control the disease is by using chemical means [3]. S. rolfsii is a pathogen of several crops and is not easy to control by conventional means [4, 5].

In the past few years, management of diseases using biological antagonists has been increasing continuously. This is influenced by the idea that they may be potential alternatives to the use of chemicals for managing the plant diseases caused by soil-borne pathogens [6-8]. It is reported that the disease intensity in the field ranges from 5-20% [9]. The pathogen is not easy to control by conventional means. The fungicides usage to control the disease is discouraged as this crop is having great importance in medicine. A small amount of pesticide residue in the preparation is not allowed. The use of bio-control agents has been employed instead of using chemicals as an alternative to control plant pathogens. *Trichoderma* strains are among the most studied fungal bio-control agents and are successfully used as biopesticides and biofertilizers in greenhouse and field plant production [10]. These applications are related to their ability to control plant diseases and to promote plant growth and development [11-14]. The strains of *Trichoderma* used as bio-control agents show different mechanisms of action in their antagonistic interactions with fungal pathogens.

The use of bio-control agents is mostly preferable to the control of pathogens on herbs those are used in cosmetic and pharmaceutical industries, by which the residual effect of pesticides can be completely avoided. The bio-control agents were found to be more effective, if they were isolated from the soils where the crop is grown [15, 16]. Hence, a survey was carried out to isolate *Trichoderma* species from *Mentha arvensis* from cultivated soils and was tested against the collar-rot caused by *S. rolfsii.*

Materials and Methods

Seven soil samples were collected from *Mentha arvensis* growing area in Central institute of medicinal and aromatic plants (CIMAP), Hyderabad, and research field are used to isolate *Trichoderma* species.

The *Sclerotium rolfsii* Sacc (SR1) causative organism of collar-rot disease of *Mentha arvensis* was isolated from infected plants collected from the research field.

Isolation and Characterization of Trichoderma Isolates

Serial dilution plate technique and soil plate method using Potato dextrose agar medium (PDA) is used to isolate *Trichoderma* colonies. The colonies obtained on agar plates were isolated, subculture and preserved on PDA for further use. *Trichoderma* isolates were identified according to the identification key based on the characters like growth rate of culture, aerial mycelium, production of distinctive, white or green condition; the color, size, shape, and ornamentation of conidia conidiogenous pustules formation; characters of chlamydospores; disposition, branching, size and shape of conidiophores and phialides [17-21].

Screening of isolates for bio-control potency against S. rolfsii

The forty-five isolates of *Trichoderma* obtained in this study were tested against the *S. rolfsii* using the dual culture technique [22]. The mycelial disc of 5mm diameter of *Trichoderma* isolates from a four-day old culture was placed one side 1cm away from the edge of the petriplate containing PDA media, and the disc of *S. rolfsii* from a four-day old culture was placed

on the opposite side 6 cm apart from the *Trichoderma* disc. The plates were incubated at **24+_2°c** for one week. The plates are observed at regular intervals for the radial growth of the pathogen and also the antagonistic *Trichoderma* isolates. The formation of *sclerotia* in the dual plate is observed and number of sclerotia formed was counted. The results are tabulated and statistics is carried out.

Testing the viability of sclerotia

The sclerotia formed in dual plate were surface sterilized using 1% Sodium hypochloride solution. The washed sclerotia were placed on 2% water agar to assess the viability of sclerotia.

Results and Discussion

The population of *Trichoderma* spp. was ranged from 2 - 8 X 10^3 CFU g^{-1} of soil. A total number of forty-five isolates of *Trichoderma* were obtained from soil samples. They belong to *T. atroviride* Karsten(2), *T. crassum* Bissett(1), *T. fertile* Bissett(1), *T. fasciculatum* Bissett(1), *T. gamsii* Samuels & Druzhinina(2), *T. harzianum* Rifai(15), *T. Hypocrea lactea*(1), *T. konigiopsis* Samuels,C.Suarez & H.C. Evans(2), *T. longibrachiatum* Rifai(2), *T. neokoningii* Samuels & Soberanis(1), *T. ovalisporum* Samuels & Schroers(5), *T. pseudokoningii* Rifai(2), *T. reesii* E.G.Simmons(1), *T. strictipilis* Bissett(1), *T. tiwanense* Samuels & M.L.Wu(1), *Trichoderma* spp (7). Of all isolates tested, 12 isolates inhibited the radial growth of *S. rolfsii* and formation of sclerotial bodies. The effective isolates belong to *T. fertile* (M3), *T. fasciculatum* (M29), *T. gamsii* (M11, M15), *T. harzianum* (M21), *T. konigiopsis* (M32, M33), *T. neokoningii* (M6), *T. strictipilis* (M28), *T. tiwanense* (M43), *Trichoderma* spp (M26), and (M35). These isolates were more aggressive in inhibiting the mycelial growth of the *S. rolfsii* and inhibition ranged from 48.5 to 58.22%. *T. fasciculatum* (M29) showed the formation of less number of sclerotia in test plate. *T. harzianum* (M21) showed maximum inhibition of test pathogen but no overgrowth was found and sclerotial formation is restricted to only one per test plate. The *T. strictipilis* (M28) showed a different antagonistic activity by over growing on pathogen but more number of sclerotial bodies was produced. *T. neokoningii* (M6), *T. gamsii* (M11, M15) showed more over growth on pathogen and 2-4 number of sclerotia were formed, with these isolates the zone of inhibition was clearly observed in test dual plate. Other isolates did not show either antagonistic effect against pathogen or reduction in the sclerotial formation. Instead the test pathogen grew over the *Trichoderma* and produced more sclerotia. Some isolates like *T. atroviride* (M48), *T. harzianum* (M13, M46, 47), *T. ovalisporum* (M17), *T.reseii* (M30) almost produced the same number of sclerotia like in control plate. It is interesting to note the uncontrollable growth of pathogen in dual plate with *T.harzianum* (M46, 47), *T. atroviride* (M48). *S. rolfsii* growth was observed out of the petriplate on opposite Where *T. harzianum* (M36, 38), *Trichoderma* spp (M34, 39) were inoculated. The study clearly indicates that different isolates of same species are having different degree of effect on the pathogen growth. The screening of all isolates in the study proved that selection of bio-control agent is always important to identify the appropriate strain. Among the effective *Trichoderma* isolates the growth of *T. harzianum* (M21), *T. fasciculatum* (M29), *T. konigiopsis* (M32, 33) growth was more and minimized the growth of pathogen after 7 days of incubation, in addition the pathogen never showed overgrowth on the *Trichoderma*.

The results showed that the number of sclerotia formed by the pathogen in dual plate was 1 to 86 and in control it was 98. The isolates which over grow on the pathogen or limiting the growth of the pathogen inhibited the sclerotial formation. Non antagonistic isolates like *T. harzianum* (M46, 47), *T. ovalisporum* (M17, 20) had little effect on the formation of sclerotia. More over when these isolates were growing over *Trichoderma,* the pathogen produced sclerotia profusely in greater numbers almost equal to control.

Table 1: 5% Level of Significance.

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	628.000	11	57.091	21.958	.000
Within Groups	156.000	60	2.600		
Total	784.000	71			

This above table represents there is no significance difference between the isolates.

In the above isolates by LSD test (1,2), (1,3), (1,4), (1,7), (1,11), (2,3), 2,7), (2,11), (3,4), (3,7), (3,9), (3,11), (4,7), (4,9), (4,11), (4,12), (5,6), (6,8), (7,9), (7,11), (8,10), (9,11), (9,12) Pairs are insignificant.

Isolate No.	Isolate Name	Radial growth (mm)
M3	*T. fertile*	45.0000±2.6818
M6	*T. neokoningii*	45.6667±1.5483
M11	*T. gamsii*	44.6667±0.5852
M15	*T. gamsii*	43.6667±1.5483
M21	*T. harzianum*	36.3333±3.0967
M26	*Trichoderma spp*	38.0000±3.5114
M28	*T. strictipilis*	44.6667±1.1704
M29	*T. fasciculatum*	39.3333±0.5852
M32	*T. konigiopsis*	43.0000±1.0136
M33	*T. konigiopsis*	40.3333±0.5852
M35	*Trichoderma spp*	44.6667±1.5484
M43	*T. tiwanense*	42.6667±0.5852

Table 2: 1% level of significance

ANOVA

Data set

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	628.000	11	57.091	21.958	.000
Within Groups	156.000	60	2.600		
Total	784.000	71			

This above table tells us there is no significance difference between the isolates

In the above isolates by LSD test (1,2), (1,3), (1,4), (1,7), (1,9), (1,11), (1,12), (2,3), 2,7), (2,11), (3,4), (3,7), (3,9), (3,11), (3,12), (4,7), (4,9), (4,11), (4,12), (5,6), (6,8), (7,9), (7,11), (7,12), (8,10), (9,11), (9,12), (10,12), (11,12) Pairs are insignificant.

The experiment was continued by testing the viability of sclerotia formed in dual plate technique. All 12 effective isolates in controlling the growth rate of the *S. rolfsii* had an effect on sclerotial viability. The results showed that except M28, all the sclerotia formed in dual plate with effective isolates are mostly non-viable. The percentage of viability is between 12.5 and 40. Most of effective antagonistic *Trichoderma* isolates invitro, suppresed the sclerotial germination and almost completely killed the sclerotia. The viability of sclerotia was more where the antagonistic effect of Trichoderma isolates was not observed.

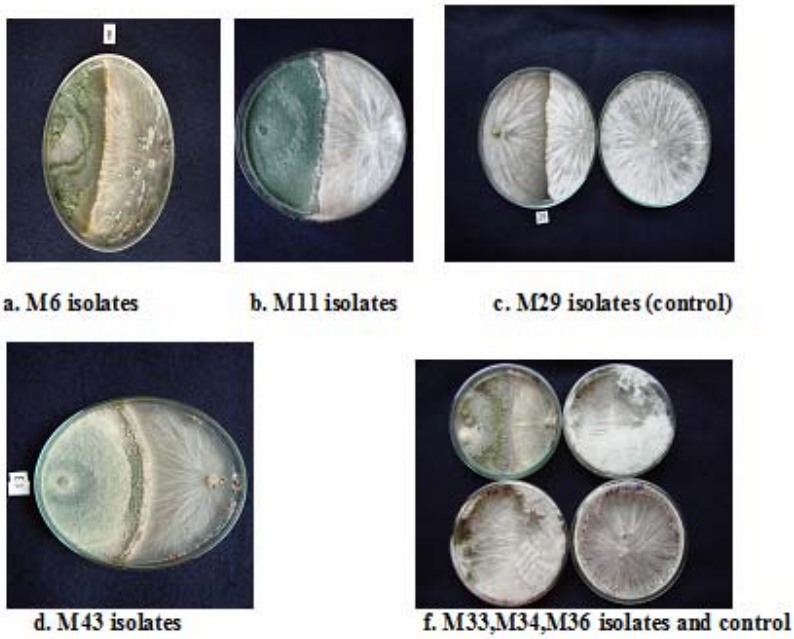

Figure 1: Dual culture photographs

Discussion

Out of forty-five isolates tested, twelve isolates are effective in controlling the growth as well as sclerotial formation. It is attributed that the release of higher extracellular enzymes in binding and lysis of *S. rolfsii* cell wall than non effective isolates. The results of Elad [23] revealed that non effective isolates of *Trichoderma* have less extracellular enzyme activity. It was found that all species were effective at different degree in arresting the growth of *S. rolfsii. Trichoderm* species are known to be highly efficient against *S. rolfsii* [24]. Biological control of plant pathogens can be highly effective especially with hyperparasitizing potentials of antagonists on pathogenic fungi. *Trichoderma* spp. completely overgrew the pathogen with percentage inhibition ranged from48.5 to 58.22% (Table I). *Trichoderma* spp are reported to release active lytic enzymes, that can digest these cell wall components of pathogen [24]. The extra cellular enzymes may play a role in biological control [25]. Similar findings on the antagonistic

parasitism of *T. harzanium* on *S. rolfisii* have been reported [23]. The use of *Trichoderma* species effectively reduced the viability of sclerotia of S. *rolfsii* [26, 27]. *T. harzianum* was found to inhibit the *in vitro* growth of *S.rolfsii* and produced coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae [28]. Non effective isolates of *Trichoderma*, the *S. rolfsii* produced more sclerotia after over growing on the *Trichoderma* isolates.This clearly indicates that, the test pathogen is under stress for food and space, hence started formation the resting bodies like sclerotia. Sclerotia absence of nonviability in test plate indicates that lysis of internal tissues of sclerotia due to antagononistic activity of *Trichoderma*. This clearly indicates that the mechanism of enzyme activity operating to distroy the viability of sclerotia.

In the dual plate, the mycelium of *T. longibrachiatum* and *T. hypocrea lactea* lysed with the test pathogen when it over grew on the *Trichoderma*. The hyphae of *Trichoderma* lysed and further growth is arrested. In addition the pathogen produced sclerotial bodies only on the area of overgrowth but not on the other place. This clearly indicates that the *Trichoderma* is highly campetative for the space and food which caused starvation of the pathogen, in turn induced the formation of sclerotial bodies. The sclerotia formed in the dual plate with *T. longibrachiatum* were nonviable than with *Hypocrea lactea*.

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