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S. Uma and P. Jeevan

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Dual Culture Effect of Marine Trichoderma Recorded from Athirampattinum Against Plant Fungi Obtained from Thirukalapatti Village Spinach Cultivating Field, Sivagangai, Tamil Nadu

S. Uma*[1] and P. Jeevan[2]

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A B S T R A C T

It was reported that yield of the spinach reduced due to the plant disease. *Fusarium* wilt, consider to be a soil borne disease which highly influence the production of crops. Outbreak of *fusarium* diseases causes major economic lose on crops throughout the world. *Fusarium oxysporum*, *Rhizoctonia solani, Pythium aphanidermatium, Fusarium culmorum, Gaeumannomyces graminisi, Sclerotium rolfsii, Phytophthora cactorum* are some of plant pathogens that cause severe diseases in various cash crops. It was reported that *fusarium oxysporum* species "Spinaciar" proven to be the most dreadful disease of the crop spinach. *Fusarium* species also causes damping-off, root rot, and discoloration of both vascular system of seedlings and mature plants. To overcome this situation biocontrol methods should be followed. It was reported that *Trichoderma* species – are recorded to an effective biocontrol agent that act against pathogens such as *Fusarium oxysporum, Rhizoctonia solani, Pythium aphanidermatium, Fusarium culmorum, Gaeumannomyces graminisi, Sclerotium rolfsii, Phytophthora cactorum, Botrytis cinerea and Alternaria* species effectively. Thus, *Trichoderma* species isolated from the coastal area Athirampattinum was tested against the fungal pathogens which are isolated from the spinach cultivating field of Thirukalapatti Village to overcome the disease and to found the potentiality of marine Trichoderma (Antagonistic) by dual culture method which was recognized to be a viable alternative method to manage plant diseases.

***Key words*:** Spinach, Marine fungi, Dual culture, Antagonistic fungi

Fungi are very successive soil inhabitant, with high plasticity and capacity to adopt to adverse condition [1]. Soil fungi play an important role as major decomposer in the soil ecosystem. They decompose the soil components by producing a wide variety of extracellular enzymes [2]. There are about 75000 species of soil fungi in the world. Fungi are one of the dominant group presents in soil, which strongly influence ecosystem structure and functioning and thus plays a key role in many ecological services. Soil borne plant "Pathogenic fungi" cause a variety of disease such as rot (stem, root, crown), damping-off and wilts. Therefore, there is a growing interest in designing biocontrol agents, its biological functioning to overcome the crop diseases and also an integrated disease management approach, including the use of disease-resistant cultivars, crop rotation, care full irrigation and organic fungicides to produce a high-quality product of crops [3].

The marine mycota is represented by lower fungi (*Haplomastigo mycotina* and *Diplomastigomycotina*) and higher fungi (*Ascomycotina*, *Basidiomycotina*, and *Deuteromycotina*). The estimated coastal isolated fungi was about 1500 species. This number seem to be low according to the number of estimated terrestrial fungi, which was estimated around 250,000 species. Several bioactive like cytoglobosins and halovirs were isolated from marine fungi. Thus, it was proved that numerous marine fungi with remarkable structures and ability to produce several bioactive compounds which are used for the production of biofertilizers [4].

* S. Uma
✉ umasivagurunathan@gmail.com

[1-2] P. G. and Research, Department of Microbiology, J. J. College of Arts and Science, (Autonomous), Pudukkottai, Affiliated to Bharathidasan University, Thiruchirapalli, Tamil Nadu, India

MATERIALS AND METHODS

1. a.) *Collection of infected leaf Sample (Spinach oleracea)*

The infected leaf sample for our study was collected from the "Spinach Cultivating Field" of "Thirukalapatty" (Village), Thirupathur, Sivagangai district.

b.) *Collection of soil sample from marine*

The marine soil samples were collected from 3 seashore areas namely Adhirampattinum, Pudhupatinum and Monara which comes under Tanjavur District.

2. a.) *Isolation of pathogen from infected leaf*

The surface sterilized explanted leaf samples were placed on "Potato dextrose agar plates". The plates were then incubated at room temperature for 4-5 days and observed for pathogen growth.

b.) *Isolation of fungi from samples*

The fungus was isolated from marine soil samples by "Serial Dilution Technique". One ml of sample from the dilution of 10^{-3} and 10^{-4} was aseptically added to sterile Petri Plates containing solidified "Potato dextrose agar medium" of twenty ml. Spread plate technique was used for the isolation of microorganisms. The plates were incubated for three days at 37°C [5].

3. *Identification of fungi*

Staining method (Lacto phenol cotton blue)

Place a drop of 70% alcohol on a clean microscopic slide. Immerse the fungus specimen obtained from cultured petri plates, in the drop of alcohol in the microscopic slide. Add one, or two drops of the lacto phenol cotton blue stain. Place a sterile coverslip above the preparation by avoiding air bubbles formation for microscopic observation. Identification of fungal taxa was based on illustrated Genera of imperfect fungi [6], Micro fungi on land plants [7].

4. *Dual culture technique*

Colony interaction of *F. oxysporum* and *T. viride* was studied on PDA containing plates by using dual culture method (Skidmore and Dickinson [7]. The growth inhibition in the colony of the test pathogen and the antagonistic fungi was calculated and interaction grade have been determined as proposed by formula [8].

Percentage growth inhibition = r – r 1/r × 100

r = radius of fungal colony without antagonist towards the center of the plate

r1 = radius of the fungus colony from centre towards the antagonistic

Fusarium species observed to be higher in number, hence consider as targeted pathogen. Here after the fusarium colony is represented by FJJC1.

Table 1 Isolation of fungi from spinach leaves collected from spinach cultivated field of "Thirukalapatti" Village, Sivagangai district

Name of the fungi	Different leaf samples				
	L_1	L_2	L_3	L_4	Total
Aspergillus oryzae	02	03	02	02	09
Aspergillus flavus	05	03	02	02	12
Alternaria alternate	03	02	03	02	10
Fusarium sp	**08**	**07**	**06**	**08**	**29**
Penicillium	04	05	03	03	15
Aspergillus candidus	-	01	02	-	03
Total number of colonies	22	21	18	17	**78**
Total number of species	5	6	6	5	

Table 2 Effect of dual culture experiments of potential micro fungi collected from Athirampattiuum against pathogen *Fusarium oxysporum (FPJJC1)*

Name of the fungi	Zone of inhibition (mm)			
	A	B	C	D
Alternaria alternata	04.3±4.01	-	-	-
Aspergillus flavus	6.02±1.36	12.3±4.01	4.05±0.04	6.03±0.06
Aspergillus fumigatus	4.21±1.03	09.3±3.11	5.07±0.03	7.08±0.04
Aspergillus luchensis	2.05±3.86	12.0±4.04	6.05±0.04	5.03±0.06
Aspergillus niger	2.21±1.55	11.3±3.56	5.04±0.05	7.06±0.07
Aspergillus ochraceus	-	-	02.4±3.11	-
Aspergillus ruberum	-	-	-	-
Aspergillus terreus	4.05±1.21	15.6±5.02	6.03±0.05	9.04±0.03
Aspergillus oryzae	3.11±1.45	09.6±2.56	6.04±1.05	8.05±0.04
Aspergillus sydowii	-	-	-	-
Aspergillus versicolor	-	-	-	-
Curvularia lunata	3.13±1.55	09.2±1.56	6.03±0.05	5.04±0.03
Helminthosporium oryzae	10.2±0.12	-	-	-
Memnoniella sp.	2.08±0.91	09.3±5.60	4.02±0.10	6.04±0.06
Penicillium sp.	3.01±1.07	12.2±5.01	6.05±0.08	6.03±0.07
Trichoderma viride	3.05±3.17	17.0±4.32	9.05±0.08	6.03±0.07

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, **D**- Colony growth of the pathogen away from the antagonistic fungi

RESULTS AND DISCUSSION

In this study, dual culture of direct methods were used to assess the ability of marine fungi isolated from the soil sample collected from different places namely Athirampattinum, Puthupattinum and Manora. Among all these areas, the fungi isolated from the Athirampattinum shows higher zone of inhibition against *Fusarium oxysporum (FPJJC1)* which causes the disease in *Spinach oleracea* plants. In invitro method it was observed that *Aspergillus flavus, A. fumigatus, A. luchensis, A. niger, A. terreus, A. oryzae, Alternaria alternate, Curvularia lunata, Helminthosporium oryzae, Memnoniella* sp, *Penicillium* sp and *Trichoderma viride* with 6.02±1.36, 4.21±1.03, 2.05±3.86, 2.21±1.55, 4.05±1.21, 3.11±1.45, 04.3±4.01, 3.13±1.55, 10.2±0.12, 2.08±0.91, 3.01±1.07 and 3.05±3.17 mm Colony growth of the antagonistic fungi towards pathogen *F. oxysporum* [9-10]. It was noted that, *Aspergillus candidus, A. flavus, A. fumigatus, A. luchensis, A. niger, A. terreus, A. oryzae, Curvularia lunata, Memnoniella* sp, *Penicillium* sp and *Trichoderma viride* with 03.0±0.12, 12.3±4.01, 09.3±3.11, 12.0±4.04, 11.3±3.56, 15.6±5.02, 09.6±2.56, 09.2±1.56, 09.3±5.60, 12.2±5.01 and 17.0±4.32mm colony growth of the antagonistic fungi away from the pathogen respectively. Whereas in the case of colony growth of the pathogen towards the antagonistic fungi *Aspergillus flavus, A. fumigatus, A. luchensis, A. niger, A. ochraceus, A. terreus, A. oryzae, Curvularia lunata, Memnoniella* sp, *Penicillium* sp and *Trichoderma viride* was 4.05±0.04, 5.07±0.03, 6.05±0.04, 5.04±0.05, 02.4±3.11, 6.03±0.05, 6.04±1.05, 6.03±0.05, 4.02±0.10, 6.05±0.08 and 9.05±0.08 mm zone of inhibition was found to be recorded against beneficial fungi [11-12]. It was also stated that *Aspergillus flavus, Aspergillus fumigatus, A. luchensis, A. niger, A. terreus, A. oryzae, Curvularia lunata, Memnoniella* sp, *Penicillium* sp and *Trichoderma viride* show the zone of inhibition from 6.03±0.06, 7.08±0.04, 5.03±0.06, 7.06±0.07, 9.04±0.03, 8.05±0.04, 5.04±0.03, 6.04±0.06, 6.03±0.07 and 6.03±0.07mm colony growth of the pathogen away from the antagonistic fungi [13-14]. Among all the micro fungi *Trichoderma viride* shows ultimate zone of inhibition when compared to all other micro fungi isolated and tested respectively.

CONCLUSION

During the study period, a total of 16 potential micro fungi were isolated from the soil sample of Athirampatinum coastal area. Fungal species were enumerated by spread plate techniques. In this study of dual culture technique, it was reported that *Trichoderma viride* species obtained from the soil sample of Athirampatinum observed to have maximum zone of inhibition against *Fusarium oxysporum* which was a plant pathogen affect the spinach crop. Thus, it was reported that the marine fungi have the potential to suppress terrestrial plant pathogen and further studies should be needed to explore the hidden potential of marine *Trichoderma* species.

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