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Evaluation of antagonistic activity of potential soil fungi against pathogen

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Abstract
The mycoflora were isolated by using soil dilution technique and soil plate technique on Potato Dextrose Agar medium supplemented by suitable antibiotics i.e streptomycin. Identification and characterization of the mycoflora were made with the help of authentic manuals of fungi. This particular study examined distribution and relative abundance of earthworms under different land use patterns and their influence on the physico-chemical properties of the soil. We measured the species composition communities across the ecosystems and effect of abiotic factors on them from various ecological regions of southern India. A simple dual culture agar plating technique has been developed and evaluated for its efficiency in determining the relationship of gut bacteria of sand fly with Leishmania donovani promastigotes. There are about fouteen morphologically distinct fungal colonies have been isolated from the gut homogenate of Phlebotomus argentipes. In dual culture method, each fungi isolate was inoculated in one half of the plate and the promastigotes of Leishmania was inculcated in the other half by poured. After incubation, the type of association was determined based on the presence or absence of promastigotes colonies. The efficient antagonistic fungi, also possessed *in vitro* multiple traits for plant growth promotion and improved rice seedling growth.

Keywords: isolation, identification, physicochemical properties and dual cuture technique

Introduction

Soils are highly complex systems, with many components playing diverse functions mainly due to the activity of soil Organisms .Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth. Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soils. Type of cultivation and crop management practices found to have greater influence on the activity of soil microflora. Continuous use of chemical fertilizers over a long period may cause imbalance in soil microflora and there by indirectly affect biological properties of soil leading to soil degradation. Fungi are fundamental for soil ecosystem functioning. Especially in forest and agricultural soils, they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization. Fungi are an important component of the soil micro biota. Micro fungi play a focal role in nutrient cycling by regulating soil biological activity. The quantities of organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil. In addition to chemical fertilizers and wide range of pesticides shows adverse effect on mycoflora which are much useful to maintain soil fertility and eco-balance in the soil atmosphere. The members and kinds of microorganisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration, pH and temperature etc. To isolate mycoflora from different crop fields, and to observe the percentage contribution of different fungal species.

The dual culture assay was standardized in terms of inoculation timing of *Streptomyces* antagonist and pathogen, and growth rate of different fungal pathogens. In case of fast-growing fungi, inoculation of the antagonist 2 or 3 days prior to the pathogen resulted in significantly stronger inhibition of mycelium growth. One hundred and thirty *Streptomyces*strains were evaluated against six destructive soil borne pathogens. The activity of strains varied from broad-spectrum to highly specific inhibition of individual pathogens. All strains inhibited at least one tested pathogen. Three strains, which combined the largest broad-spectrum with the highest inhibition activity, were selected for further characterization with four vegetable species. All of them were able to colonize seed surface of all tested vegetable crops. They mostly improved radicle and hypocotyl growth *in vitro*, although no statistically significant enhancement of biomass weight was observed *in vivo*. Occasionally, transient negative effects on germination and plant growth were observed.

We performed the screening of bacteria isolated from soil as potential antagonists against the dirty panicle disease fungal pathogens of rice. The effect of the antagonistic bacterial strain and its extracellular metabolites on the pathogenic fungi were studied *in vitro* using various methods. The antifungal compounds produced by BAS23 were isolated using reversed-phase high performance liquid chromatography (RP-HPLC) and analyzed using electrospray ionization-mass spectrometry (ESI–MS) and nuclear magnetic resonance spectroscopy (1 H NMR). In addition, plant growth-promoting activity and its effect on root and shoot lengths of rice *in vitro* were also reported. To our knowledge, this is the first report of this antagonistic bacterium displaying broad spectrum activities against the dirty panicle rice fungal pathogens, C. lunata, H. oryzae and F. semitectum, and also promoting rice seedling growth.

The important pathogens causing post-harvest diseases of tomato are *Alternaria, Aspergillus, Rhizopus,* etc. which

make the fruit not only to lose its appearance but also make them to become soft and watery (Ratnam and Nema, 1967) [11]. Biological control of plant pathogens through microorganisms has been considered as a potential tool for management of post–harvest diseases in recent years and search for potential bio-agents has been increased (Balai and Singh, 2013). *Trichoderma* spp. are now the most common fungal bio-control agent that have been comprehensively studied and deployed throughout the world. The bio-efficacy of antagonists and their culture filtrate in management of Rhizopus rot of tomato incited by *Rhizopus oryzae.*

Materials and Methods

Isolation of Soil Mycoflora

Dilution plating technique described by Warcup (1950) [12] was used to isolate the fungi from Tomato field soils. One g soil sample was weighted and diluted in 10 ml distilled water. One ml of the diluted (10^{-3}, 10^{-4} and 10^{-5}) sample was poured in separate Petri plates, then sterilized PDA medium was poured into the Petri plates, tilt gently and allow for solidification. After solidification the inoculated plates were incubated in a dust free cupboard at the room temperature (24±2 °C) for 7 days. Replicates for each dilution were maintained. One per cent streptomycin solution was added to the medium before pouring into Petri plates for preventing fungal growth.

The colonies growing on PDA plates with different colour and morphology were counted separately. A portion of the growing edge of the colony was picked up with the help of a pair of needles and mounted on a clean slide with lacto phenol cotton blue stain. The slide was gently heated in a spirit lamp so as to facilitate the staining and remove air bubbles if any. The excess stain was removed with the help of tissue paper and then the cover slip was sealed with transparent nail polish. The slide was observed under a compound microscope.

Microphotography of the individual fungal species was also taken using Olympus light microscope, Thanjavur.

Identification of soil fungi

The fungi were identified by using standard manuals such as A manual of soil fungi (Gillman, 1957), *Dematiaceous Hyphomycetes* (Ellis, 1971) [14], More Dematiaceous Hyphomycetes (Ellis, 1976) [15] and Hyphomycetes (Subramanian, 1971), Manual of *Aspergillus* (Raper and Fennell, 1965) and Manual of *Penicillia* (Raper and Thom, 1949) [16].

Dual Culture Technique (Skidmore and Dickinson, 1976)

Colony interaction of *Helminthosporium oryzae* and the soil fungi were studied using dual culture experiments. The test organisms and soil fungi namely *Aspergillus flavus*, *Aspergillus awamori*, *A.terreus*, *A. Niger*, *A. luchensis*, *Curvularia lunata*, *Memonmila* sp., *Penicillium* sp and *Trichoderma* sp. were grown separately on PDA medium. Then agar blocks were cut from actively growing margin of the individual species of soil fungi and test organisms inoculated test just opposite to each other approximately 3 cm apart. Three replicates and respective control for each set were recorded at 24 hrs intervals. The percentage inhibition of growth of pathogen was calculated. Assessment was made when the fungi had achieved an equilibrium after which there was no further alternation in the growth. Since both of the organisms were mutually inhibited, the assessment was made for both organisms.

$$\text{Percentage of inhibition growth} = \frac{r - r_1}{r} \times 100$$

r = growth of the fungus from the centre of the colony towards the centre of the plate in the absence of antagonistic fungi.

r^1 = growth of the fungus from centre of the colony towards the antagonistic fungus.

The colony interactions between the test pathogen and the soil fungi were assessed by the following model proposed by and Dickinson and Broadman, (1971) [18]. Five types of interaction grades as proposed by Skidmore and Dickinson, (1976) [11] were used. They are as follows:

Grade 1: Mutual intermingling growth without any macroscopic sight of interaction.

Grade 2: Mutual intermingling growth, where the growth of the fungus is ceased, and is being over grown by the opposed fungus.

Grade 3: Intermingling growth where the fungus under observation is growing in to the opposed fungus either above or below.

Grade 4: Slight inhibition of both the interacting fungi with a narrow demarcation line (1-2 mm).

Grade 5: Mutual inhibition of growth at a distance of > 2 mm.

Results and Discussion

The fungi isolated from the paddy field soil sample. Totally isolated from the colonies is more than 25. They are growing from PDA medium. The isolated colonies is identified from fungal staining is easily observing for fungi. The specific stain if fungi is always used from Lactophenol cotton blue. The fungal colonies is clearly visible from structure.

Table 1: Isolation and identification of fungi from paddy field soil samples of Thanjavur district

S. No	Name of the fungi	Different places		
		Thiruvaiyar	Kadukava,	Ammapetti
1	*Aspergillus awamori*	-	07	-
2	*A.luchuensis*	04	-	02
3	*A.candidus*	02	02	-
4	*A.chevalieri*	05	06	05
5	*A.flavipes*	02	-	03
6	*A.niger*	15	13	12
7	*A.terreus*	11	14	10
8	*A.flavus*	14	15	13
9	*A.fumigatus*	10	13	09
10	*Curvularia lunata*	03	04	03
11	*Fusarium oxysporum*	08	14	10
12	*F.solani*	05	13	09
13	*Helminthosporium oryzae*	02	04	02
14	*Penicillium citrinum*	10	06	11
15	*P.janthinellum*	09	08	04
16	*Trichoderma* sp.	05	03	06
Total number of colonies		105	129	99
Total number of species		15	15	14

Various factors are responsible for the fungal diversity. The common ungi found *Aspergillus, Rhizopus and Fusarium.* A

fungal species of belonging to the phyla ascomycota, and genera Aspergillus (Eurotium) were successfully identified after staining with lactophenol cotton blue based on their morphological characters and microscopic analysis. Rests of the strains were (Waksman SA and Fred EB, 1922). Not identified owing to the lack of sporulating structures under present incubation conditions. On PDA *Aspergillus* colonies attained 5.5cm in diameter in seven days, with white to pale yellow margin and black conidial heads. Conidiophores attained a length upto 3.0 cm and width of 15.0 cm and were observed with white to pale walled with hyaline at the base which became brownish at the apex. (Boer W, Folman LB, Summer sbell RC and Boddy L 2005) [19] The physic chemical compounds is followed from the soil content. They are analysed from various ingredients in the soil nutrients. According to Thanjavur parameter like is pH 7.92, Electrical conductivity (dsm^{-1}) 0.42, Organic Carbon 0.23 (%), Organic Matter 0.56 (%), Available Nitrogen 110.1 (mg/kg), Available Phosphorus 4.53 (mg/kg), Available Potassium 120.3 (mg/kg), Available Zinc 0.81 (ppm), Available Copper 0.41 (ppm), Available Iron 4.00 (ppm), Available Manganese 2.01 (ppm). Calcium 11.7 (mg/kg), Magnesium 7.4 (mg/kg), Sodium 1.23 (mg/kg), Potassium 0.15 (mg/kg), Cat ion Exchange Capacity 22.0 (C. Mole $Proton^{+}$/kg), the second parameter is Kadukaval soil analysed from pH 7.91, Electrical conductivity 0.26 (dsm^{-1}), Organic Carbon 0.16 (%),Organic Matter 0.32 (%), Available Nitrogen 97.8 (mg/kg), Available Phosphorus 4.00 (mg/kg), Available Potassium 125 (mg/kg), Available Zinc 1.02 (ppm), Available Copper 0.52 (ppm), Available Iron 4.62 (ppm), Available Manganese 1.84 (ppm), Calcium 11.3 (mg/kg), Magnesium 6.5, (mg/kg), Sodium 1.29 (mg/kg), Potassium 0.23 (mg/kg), Cat ion Exchange Capacity (C. Mole $Proton^{+}$/kg), 28.6, and third parameter of Ammapettai is analysed the compounds pH, 7.88, Electrical conductivity 0.43 (dsm^{-1}), Organic Carbon 0.22 (%), Organic Matter 0.65 (%), Available Nitrogen 114.6 (mg/kg), Available Phosphorus 3.65 (mg/kg), Available Potassium 114.6 (mg/kg), Available Zinc 0.87 (ppm), Available Copper 0.45 (ppm), Available Iron 4.23 (ppm), Available Manganese 2.84 (ppm), Calcium 12.1 (mg/kg), Magnesium 7.6 (mg/kg), Sodium 1.20 (mg/kg), Potassium 0.15 (mg/kg), Cat ion Exchange Capacity 22.8 (C. Mole $Proton^{+}$/kg), analysed from the paddy field soil sample were analysed.

Table 2: Physico – chemical parameters of paddy fields soil samples

S. No.	Physico chemicals Parameters	Different places		
		Thiruvaiyaru	Kadukaval	Ammapettai
1.	pH	7.92	7.91	7.88
2.	Electrical conductivity (dsm^{-1})	0.42	0.26	0.43
3.	Organic Carbon (%)	0.23	0.16	0.22
4.	Organic Matter (%)	0.56	0.32	0.65
5.	Available Nitrogen (mg/kg)	110.1	97.8	114.6
6.	Available Phosphorus (mg/kg)	4.53	4.00	3.65
7.	Available Potassium (mg/kg)	120.3	125	114.6
8.	Available Zinc (ppm)	0.81	1.02	0.87
9.	Available Copper (ppm)	0.41	0.52	0.45
10.	Available Iron (ppm)	4.00	4.62	4.23
11.	Available Manganese (ppm)	2.01	1.84	2.84
12.	Calcium (mg/kg)	11.7	11.3	12.1
13.	Magnesium (mg/kg)	7.4	6.5	7.6
14.	Sodium (mg/kg)	1.23	1.29	1.20
15.	Potassium (mg/kg)	0.15	0.23	0.15
16.	Cat ion Exchange Capacity (C. Mole $Proton^{+}$/kg)	22.0	28.6	22.8

The soil physico-chemical properties of three places is analysed from paddy field soil are summarized. Mean values of soil pH in upland fields were lowest at U1 (8.54), medium at U2 (8.99), and highest at U3 (9.32). In paddy soil, the mean pH values were lowest at P1 (8.22), medium at P2 (9.00) and highest at P3 (9.10). These trends (in both upland and paddy fields) were positively related with the values of ESP in different fields, as the soil of U1 had the lowest ESP value of 5.42%, and U3 had the highest value of 15.73%. In paddy fields, the lowest ESP was recorded at P1 (5.85%) and the highest at P3 (13.43%). When comparing different soil layers, it was found that topsoil showed lower pH, ESP and BD than the subsoil in both upland fields and paddy fields. For upland fields, EC in topsoil was lower than that in subsoil. In comparison, for paddy fields, EC in topsoil was greater than the subsoil.

The antagonist species competed successfully with the pathogens for space and nutrients. The radial growth of the pathogen was much lesser in the *Helminthusporium oryzea* inoculated plates. This finding supports the observation of earlier reporter. Among the six antagonists used *Helminthusporium oryzea* was found to be best in radial growth inhibition and distinct inhibition zone was recorded against all five rice pathogens. This fungal antagonist showed maximum of 15mm. inhibition zone against *Helminthusporium oryzea*. The occurrence of inhibition zone between some of the antagonists and the rice pathogens could be considered as a result of the production of growth and hyphal interference may also attribute to the occurrence of inhibition zone between two fungi on dual culture plates. Mutual growth of two fungi in dual cultures is also possible when both microbes show equal growth rate, equal competition and equal capacity of tolerance to toxins produced by each of them. The overgrowth is achieved when one fungal species exhibits higher growth rate, higher capacity of toxin metabolites production and more tolerance capacity against metabolites produced in comparison to other ones. This explanation was put forwarded by previous workers, which probably holds true to explain the results obtained in the present investigation. Culture filtrates of the fungi tested in this study showed inhibitory effect on the growth of the pathogen. Growth inhibition was found to increase with the period of incubation. *T.harizanum* culture filtrate showed maximum percentage of inhibition at 15% while *T.Viride* and *T.lignorum* filtratesproduced 25% inhibition, respectively.

Plate 3: Effect of antagonistic fungi on the growth of *Helminthosporium oryzae* by *invitro* method

S.No.	Name of the fungi	A	B	C	D
1.	*Aspergillus awamori*	5.03±0.47	09.3±2.04	4.03±1.06	6.60±1.45
2.	*Aspergillus flavus*	11.1±1.04	18.5±3.08	6.06±3.02	10.0±3.66
3.	*A.fumigatus*	6.02±0.37	08.3±3.04	5.05±0.06	7.66±1.55
4.	*A.terreus*	7.05±2.84	11.0±2.05	5.07±0.04	9.00±2.33
5.	*A. niger*	8.05±2.19	13.0±3.31	6.05±0.08	10.10±1.33
6.	*A. luchensis*	4.05±0.24	10.5±4.00	2.03±0.05	7.06±3.86
7.	*Curvularia lunata*	5.04±0.20	09.3±3.10	4.02±0.06	6.01±1.07
8.	*Memonmila* sp.	6.08±0.89	12.2±5.20	4.12±0.10	8.91±1.97
9.	*Penicillium* sp	4.07±0.73	11.3±4.22	3.04±0.12	6.08±1.50
10.	*Trichoderma* sp.	5.21±0.50	14.3±3.46	5.04±0.05	7.97±0.91

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 Culture filtrates of the fungi tested in this study showed inhibitory effect on the growth of the pathogen. Growth inhibition was found to increase with the period of incubation. *Aspergillus flavus*, *A.fumigatus*, *A.terreus*, *a. Niger*, *Memonmila* sp., *Penicillium* sp. and *Trichoderma* sp. culture filtrate showed maximum percentage of inhibition at 25% *A.terreus* and *Memonmila* sp., while filtrates produced 05% inhibition *Penicillium* sp., respectively.

In dual culture both the fungi grow simultaneously without any hinderance with each other upto 4 days but 5th day onwards it was observed that the mycelia of *Penicillium chrysogenum* was unable to penetrate the *Trichoderma viride* mycelia. The results obtained showed that percent inhibition of mycelial growth was 55.67% against *Penicillium chrysogenum* (.After 7days onwards it was found that the hyphal tips of *Penicillium chrysogenum* become disorganised and disassociated. Finally, the hyphal tips of *Penicillium* were decomposed and get distorted without any further growth Etabarian (2006) reported *Trichoderma viride* prevent growth of the colony area of *Macrophomina phaseoli* by 19.2% and 34.9% using the dual culture and cellophane methods respectively. Henis *et al*., (1983) reported that the different isolates of *Trichoderma* parasitized *S. Rolfsii* with varying percentages of inhibition. Dharmaputra *et al*, (1994) tested two isolates of *T. harzianum* and oneisolate of *T. viride* against *Ganoderma* spp. and found that all isolates inhibited the mycelial growth of thepathogen, but *T. harzianum* showed the best performance. Most other studies also reported that *T. Viride* and *T. harzianum* were the most effective growthinhibitors of plant pathogens (Poddar *et al.,* 2004; Lane & Bowen 2005; Dubey *et al*., 2007; Hajieghrari *et al.* 2008).In this study, the interaction between *Trichoderma viride* and *Penicilium chrysogenum* revealed *Trichoderma* sp.penetrated the *Penicilium* sp. through apressoriastructures without coiling around it. Similar result was found by Monteiro *et al*., 2010 against *Fusarium* sp.

Table 4: Effect of culture filtrate r of antagonistic fungi against *Helminthosporium oryzae*

S.No.	Name of the fungi	Growth measurement (mm)				
		100µl	200 µl	300 µl	400 µl	500 µl
1.	*Aspergillus flavus*	3.15±2.09	2.10±1.03	1.07±0.98	0.00±0.00	0.00±0.00
2	*A.fumigatus*	2.10±1.10	1.15±1.09	0.10±0.03	0.00±0.00	0.00±0.00
3.	*A.terreus*	2.20±1.03	1.06±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.	*A. niger*	2.13±1.06	1.05±0.98	0.00±0.00	0.00±0.00	0.00±0.00
5.	*Memonmila* sp.	2.23±1.05	1.14±0.78	1.05±0.00	0.00±0.00	0.00±0.00
6.	*Penicillium* sp.	2.05±1.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7.	*Trichoderma* sp.	2.25±1.15	1.20±1.10	0.95±0.50	0.00±0.00	0.00±0.00

All the fungi tested in this study exhibited antagonistic activities against sheath rotfungi *Sarocladium oryzae*. Radial growth of the pathogen was considerably hindered by all the testantagonists under the conditions of this study. *T. harizanum* was the most antagonistic and inhibited theradial growth of the pathogen most while *T.album* was the least antagonist.

Conclusion

The present study undertaken to comprehend the hyphomycetous fungal diversity .Diversity was found to be higher in the agricultural fields and garden soils as compared to the unattended barren land. This indicates utilization of superior quality of soils for plantations. The studies also suggest that agricultural soil samples and especially the garden soil need amendment. IT has been reported by several investigators.

That symptoms of some diseases could be reproduced by the action of the culture filtrate on the host plant, instead of using the infecting spores.

References

1. Chiang CN, Soudi B. Biologie du sol et cycles biogéochimiques. In: El Hassani TA. and Persoon E (Eds), Agronomie Moderne, Bases physiologiques et agronomiques de la production végétale 1994, 85-118.
2. Kiran Singh, Jaishree Borana and Sobha Srivastava, VA., Journal of Soil Biology and Ecology,1999:19:11-14.
3. Mc Gill WB, Cannon KR, Robertson JA, Cook FD. Canadian Journal of Soil Science,1980:66:1-19.
4. Manickam TS, Venkataraman CR. Madras Agricultural Journal,1972:59:508-512.
5. Warcup JH. Trans Br Mycol Soc,1951:34:376-399.

6. Ainsworth GC, Bisby GR. Dictionary of the fungi, Commonwealth Mycological Institute Kew, Surrey,1995,445.
7. Christensen, M., A view of fungal ecology. *Mycologia*,1989:81:1-19.
8. Arunachalam KM, Arunachalam RS, Tripathi, Pandey HN, Trop Ecol,1997:38:333-341.
9. Andrea Kunova, Maria Bonaldi, Marco Saracchi, Cristina Pizzatti, Xiaoyulong Chen, Paolo Cortesi. Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth BMC Microbiology,2016:16:272.
10. Balai LP, Singh RB. Integration management of Alternaria blight of pigeon pea with some fungicides and antagonists in pot condition. The Bios can,2011:8(3):881-886.
11. Ratnam CV, Nema KG. Studies on market diseases of fruits and vegetables. Andhra Agric. Journal. 1967:14:60-65.
12. Warcup JH. The Soil-Plate Method for Isolation of Fungi from Soil, *Nature*,1950:166:117-118.
13. Gilman JC. A Manual of Soil Fungi, 2nd edn. London: Constable,1957.
14. Ellis MB. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute: Kew, Surrey, UK,1971.
15. Ellis MB. More Dematiaceous Hyphomycetes. Common wealth Mycological Institute: Kew, Surrey, UK,1976.
16. Raper KB, Thom C. A manual of the penicillia**.** The Williams & Wilkins Company, Baltimore, Porter, C.L., *Am. J. Bot*,1949:1924(11):168-188.
17. Skidmore AM, Dickinson CM. Trans.Br. Mycol, soc, 1976:66:57-6.
18. Dickinson CH, Broadman F. Trans. Br. Mycol. Soc, 1971:55:293-305.
19. Boer W, Folman LB, Summerbell RC, Boddy L. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Review, 2005:29(4):795-811.
20. Waksman SA, Fred EB. A tentative outline of the plate method for determining the number of micro-organisms in the Soil. *J. Soil Sci*,1922:14(1):27-28.
21. Etabarian HR. Evaluation of *Trichoderma* isolatesfor biological control of charcoal stem rot in meloncaused by Macrophomina phaseolina, J. Agric. Sci. Technol,2006:8:243-250.
22. Dharmaputra OS, Purba RY, Sipayung A. Researchactivities on the biology and control of *Ganoderma*at SEAMEO BIOTROP and IOPRI Marihat. In: Proceedings of First International Workshop on Perennial Crop Diseases Caused by *Ganoderma*. Ed. M. Holderness. Universiti Pertanian Malaysia, Selangor,1994.
23. Henis Y, Adams PB, Lewis JA, Papavizas GC. Penetration of sclerotia of Sclerotium rolfsii by Trichoderma spp. Phytopathology,983:73:1043-1046.
24. Monteiro VN, Nascimento Silva R, Steindorff AS, Costa FT, Noronha EF, Ricart CAO *et al* New insights in Trichoderma harzianum antagonism of fungal plant pathogens by secreted protein analysis. Curr Microbiol,2010:61:298-305.