INVESTIGATION OF B. BASSIANA PERSISTENCE AND VIRULENCE FACTOR AGAINST P. XYLOSTELLA OF PEATLAND IN CENTRAL KALIMANTAN

by Noor Aidawati

Submission date: 13-Aug-2024 11:39PM (UTC-0400)
Submission ID: 2431806568
File name: Trichoderma_spp._from_tidal_swamp_lands_of_South_Kalimantan.pdf (450.71K)
Word count: 3440
Character count: 19225

IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 3 Ver. II (Mar. 2016), PP 40-44
www.iosrjournals.org

Genetic diversity of *Trichoderma* spp. from tidal swamp lands of South Kalimantan

Suparno[1], Sukoso[2], Luchman Hakim[3], Noor Aidawati[4]

[1]*Graduate School for Environmental Science, Brawijaya University Malang East Java and Institute of Plant Protection and Horticulture, Mekatani Guntung Manggis, Banjar Baru 70721, South Kalimantan, Indonesia*
[2]*Faculty of Fisheries and Marine Science, Brawijaya University, Malang East Java Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, East Java, Indonesia*
[4]*Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia*

Abstract: *Tidal swamp lands in South Kalimantan, Indonesia, has high biodiversity content, including Trichoderma which are potentially become bio-control agent in integrated pest management. The exploration of Trichoderma biodiversity, therefore, was important. This study aims to identify and describes the genetic diversity of Trichoderma isolates from South Kalimantan, Indonesia. The sample of Trichoderma with codes BM (Banjarmasin), KB (Kota Baru), TB (Tanah Bumbu), B (Banjar), BK (Barito Kuala) and TL (Tanah Laut) has similarity about 100 % with Trichoderma asperellum strain ZWPBG1 (KR868286.1). This sample also has 98.76% similarity with Trichoderma atroviride voucher TriAtv JSB123 (KC569357.1), Trichoderma viride strain ATCC 28038 (AY380909.1) and Trichoderma Vinosum strain GJS 99-158 (AY380904.1). The sample has similarity with Trichoderma harzianum voucher 60693 (KC569343.1), Trichoderma harzianum voucher TriH JSB301 (KC569359.1), Trichoderma harzianum TriH JSB971 (KC569354.1) about 91.75%. Compared to the Trichoderma virens strain PUXX-FS22 (KR296891.1), samples has similarity about 91.99%.*

Keywords : *genetic diversity, indigenous fungi, biodiversity conservation*

I. Introduction

Recent issues of rice cultivation and yield improvement are facing serious problems regarding plant pest and diseases. Biological control of soils pathogens by the addition of antagonist organism has been implemented in sustainable agriculture practices. This practice reduces the application of chemical material to eliminated pathogens in soil systems. Many species are able to parasitizing individual pathogens and therefore offer opportunities for bio-control practices. In integrated pest management program, the use of organism with its ability as bio-control is important. *Trichoderma* is one of the potential agents in integrated agriculture management [1] [2]. It is especially important in fungal diseases caused by *Rhizoctonia solani*. This species is a basidiomycete fungus which is found widely in soils. It is also found in roots systems of plants. The impact of *Trichoderma* colonization on roots has been widely reported. This fungi colony in roots has been reported contributes in root growth and development, and therefore lead to the crop productivity [3] [4].

Species diversity is an important aspect in organism conservation, including *Trichoderma*. Biodiversity provides potential sources for plant genetic improvements and provides opportunities for organism manipulation through biotechnology. Species diversity has been reported important in diseases mitigation, especially from the perspectives of organism. Study about species diversity in genetic levels provides valuable information for numerous organism aspects, and it has been claimed provides significant tools to support decisions making in conservation programs. In agricultural field, the important aspect of genetic diversity research and studies has been reported numerous, ranging from yield improvement to pest and diseases protections. These studies widely implemented in western and modern countries. In developing countries, however, attempt to map and describes species diversity in molecular levels has been implemented, but there number of study should be increased in order to understand the genetic levels of species. It is particularly important in agricultural fields [5] [6] [7].

The study of species pattern and genetic diversity among *Trichoderma* has been done in some region in the earth. Scholars notes that *Trichoderma* from the river of Danube National Park in Austria was diverse. About twenty-one strains were identified as *T. harzianum*, thirteen sample was identified as *T. rossicum*, four sample was identified *T.cerinum*, two sample was identified as *T. hamatum*, and one sample was identified as *T. atroviride* and *T. koningii*. From this study, two species was considered new taxon of *Trichoderma* [8]. Genetic diversity analysis of *Trichoderma* provides opportunities to understand the diversity pattern which area important for further bio-control development. The *Trichoderma* genetic resource is an important source for sustainable agriculture management and it is reported contributes to the food security [9] [10] [11].

The diversity of *Trichoderma* from soils of particular area provides opportunities for further management of sustainable agriculture, especially in the development of bio-control agent to countermeasure plant diseases. Scholars point out that the intensive research about fungi in tropical regions confirm that the area was rich in term of fungi which area important for future medical, agriculture and other biotechnology industry [7]. The humid and wet environment provide potential habitat for fungi to grows, and therefore it is reasonable to found numerous fungi. There are some species newly found. Mostly are collected from remotes and unstudied area in tropical regions.

The tidal swamp land in South Kalimantan, Indonesia was abundance and it is especially important for rice production development programs. The wide area of tidal swamp land in South Kalimantan provides opportunities to increase paddy rice production. In order to accelerate the paddy rice production, soil health and mitigation of plant pest and diseases were important. The tidal swamp forest of South Kalimanan has potential *Trichoderma* species. In order to identify the potentiality of indigenous *Trichoderma* resources, the species differences in morphology and growth characters has been implemented. There is, however, several limitation to identify the difference in morphology which are lead the poor recognition about *Trichoderma*. The study of *Trichoderma* genetic diversity was rare. The aims of the research are to identify the genetic diversity of *Trichoderma* spp from tidal swamp land in Kalimantan. It is especially important for plant disease management.

II. Methods

Materials

About six sample of *Trichoderma* spp from south Kalimantan was sampled and processed to the laboratory for further genome analysis. Soil sample was coded as BM (Banjarmasin), KB (Kota Baru), TB (Tanah Bumbu), B (Banjar), BK (Barito Kuala) and TL (Tanah Laut). From the field, *Trichoderma* spp was collected from soils by soil dilution plate methods. Soils suspension containing *Trichoderma* were prepared for further serial dilution and plated into PDA medium. All cultures were incubated until sporulation occurs. In order to produce single-spore culture of sample, the *Trichoderma* colony purification was done by sub-culturing conidia. The individual conidia strains were collected and stored in laboratory for further analysis. These steps were done following standard methods.

Methods

Sample Preparation

Initial methods was started by preparing culture media for growing fungi, culture transfer, isolation of pure culture, and harvesting fungi materials for genome analysis. Six sample specimen of *Trichoderma* spp from different location was grown in Potato Dextro Agar (PDA). Potato Dextrose Agar media consists of potato infusion and dextrose which are become important media for the cultivation of fungi, including *Trichoderma* spp. The isolated strains were then harvested and washed culture collection, and representative samples was collected for genetic analysts. DNA was extracted using Doyle and Doyle Methods [12].

PCR Amplification And Sequencing

The amplification of ITS5 and 4 was performed in a 15 μl which area containing 2 μl ddH2O, 1 μl primer ITS5 (10μM), 1 μl primer ITS4 (10μM), 5 μl 2xKapa 2G ready mix and 1 μl sample of DNA. Primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used in amplification. The amplification performed as within 35 cycles of pre-denaturation at 94 °C, 5 minutes; denaturation at 94 °C, 30 second, annealing at 55 °C, 30 second, extension at 72 °C, 60 second and a final extension period at 72 °C, 10 minutes. The PCR products were run in Agarose gels and the result was observed under UV trans-illuminator. Result of band pattern was documented using Canon DSLR 1100 camera. Fore sample sequencing, the ITS5 and 4 were sequenced by dideoxynucleotide method using automated DNA sequencer [12].

Data Analysis

The PCR product was then sequenced in Firstbase-Malaysia and data was read using software ABI sequencer Scanner. Sequence data was copied and stored in fasta format. The database of *Trichoderma* from NCBI was copied and stored in fasta as reference. File of sample and references was analyzed and phylogenetic trees was constructed using MEGA 5.03 software and maximum likelihood methods with kimura-2 parameters using 1000 times bootstrap.

III. Results and Discussions

Trichoderma sample with code BM (Banjarmasin), KB (Kota Baru), TB (Tanah Bumbu), B (Banjar), BK (Barito Kuala) and TL (Tanah Laut) were successfully amplified using ITS-4 primers (5′-TCC TCC GCT

TAT TGA TAT GC-3′) and ITS-5 primer (5′-GGA AGT AAA AGT CGT AAC AAGG-3′) and produce amplicons with length 590 bp (Fig.1).

The primers ITS 4 and ITS 5 are able to amplify all of the isolates, especially in ITS (Internal Transcribed Region) of ribosomal DNA (590 bp fragment). ITS region has been widely used to indentify fungi. Scholar point out that the Nuclear ribosomal internal transcribed spacer region in fungal genome is the universal DNA barcode marker which are widely used to identify Fungi [13] [14].

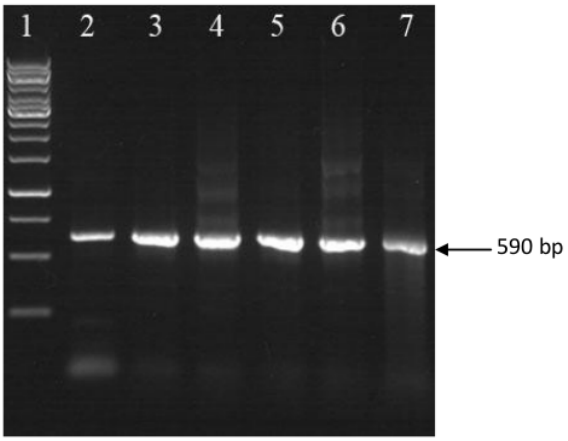

Fig.1. Profiles of amplified DNA sample from Trichoderma isolates using ITS5 and ITS4 primers. (Notes :1. Marker, 2. BM (Banjarmasin), 3. KB (Kota Baru), 4.TB (Tanah Bumbu), 5. B (Banjar), 6. BK (Barito Kuala), 7.TL (Tanah Laut)

The phylogenetic tress of *Trichoderma* isolates was shown in Fig. 2. The *Trichoderma* genes characterization revealed the variability among isolates from South Kalimantan. Based on the Fig.2, the sample of *Trichoderma* with codes BM (Banjarmasin), KB (Kota Baru), TB (Tanah Bumbu), B (Banjar), BK (Barito Kuala) and TL (Tanah Laut) has similarity about 100 % with *Trichoderma asperellum strain ZWPBG1* (KR868286.1). *Trichoderma asperellum* is the filamentous fungus which area often found and isolated from root-free soils. It is also often found in rhizomatous plants. Scholars point out that *Trichoderma asperellum* was found in soil litter and dead wood [15] [16].

This examined sample also has 98.76% similarity with *Trichoderma atroviride* voucher TriAtv JSB123 (KC569357.1), *Trichoderma viride* strain ATCC 28038 (AY380909.1) and *Trichoderma vinosum* strain GJS 99-158 (AY380904.1). Scholars point out that *Trichoderma atroviride* was found in soil from tropics to temperate zone. *Trichoderma atroviride* widely used as biocontrol to inhibits pathogenic fungi such as *Botrytis cinerea and Rhizoctonia solani*. *Trichoderma viride* was used widely as bio-control in integrated pest management programs [16] [17].

The sample of *Trichodema* which are isolated from South Kalimantan has similarity with *Trichoderma harzianum* voucher 60693 (KC569343.1), *Trichoderma harzianum voucher TriH JSB301* (KC569359.1), *Trichoderma harzianum* TriH JSB971 (KC569354.1) was about 91.75%. Compared to the *Trichoderma virens strain* PUXX-FS22 (KR296891.1), samples has similarity about 91.99% (Fig.2).

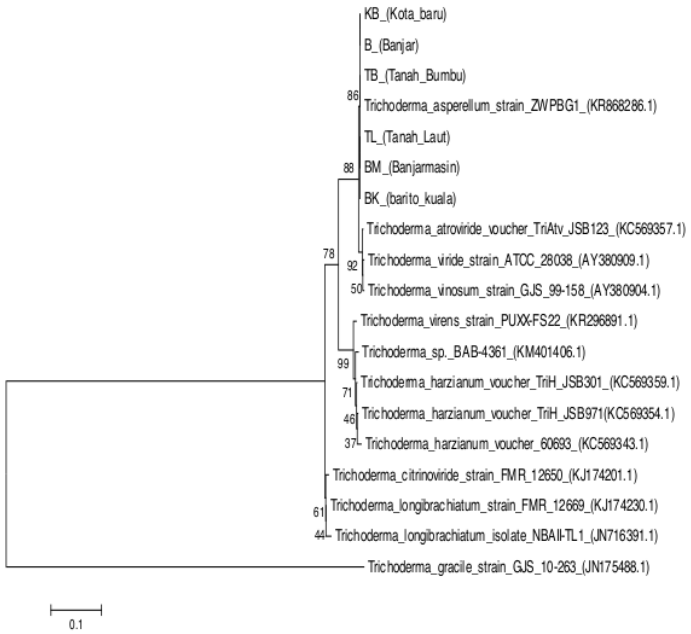

Fig 2. Phylogenetic tree of *Trichoderma* samples

Matrix of pairwise distance sample of *Trichoderma* with BM (Banjarmasin), KB (Kota Baru), TB (Tanah Bumbu), B (Banjar), BK (Barito Kuala) and TL (Tanah Laut) using *Trichoderma* references *NCBI* was shown in Table 1. In Table 1, each isolates was compared with references and shows its genetic distance. This conform the status of samples compared to the identified sample references in genes bank. This method is valuable to identify the unidentified isolated sample.

Trichoderma spp widely used as a biocontrol agent to countermeasure pathogens such as *Pythium* spp, *Rhizoctonia* spp, *Sclerotium rolfsii*, *Phytophtora palmivora* and *Fusarium* spp which are able to attack plant [18]. *Trichoderma* are able to provide direct disturbance to plant's pathogens and significantly become important competitor of pathogens to absorb nutrients and habitat of pathogens. *Trichoderma* spp are able to uses numerous substrate resources to support cell and individual growth and its resistance to chemical material. *Trichoderma* also able to produce enzymes and secondary metabolites [8]. The secondary metabolic of *Trichoderma* has potentiality to inhibits pathogens.

Table 1. Genetics distance of *Trichoderma* sample using NCBI data base references

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
TL (Tanah_Laut)																		
KB (Kota_baru)	0.000																	
TB (Tanah_Bumbu)	0.000	0.000																
B (Banjar)	0.000	0.000	0.000															
BK (barito_kuala)	0.000	0.000	0.000	0.000														
BM (Banjarmasin)	0.000	0.000	0.000	0.000	0.000													
T. asperellum strain ZWPBG1 (KR868286.1)	0.000	0.000	0.000	0.000	0.000	0.000												
T. longibrachiatum strain FMR 12669 (KJ174230.1)	0.075	0.075	0.075	0.075	0.075	0.075	0.075											
T. citrinoviride strain FMR 12650 (KJ174201.1)	0.082	0.082	0.082	0.082	0.082	0.082	0.082	0.006										
T. longibrachiatum isolate NBAII-TL1 (JN716391.1)	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.010	0.017									
Trichoderma sp. BAB-4361 (KM401406.1)	0.080	0.080	0.080	0.080	0.080	0.080	0.080	0.002	0.064	0.073								
T. harzianum voucher TriH JSB301 (KC569359.1)	0.080	0.080	0.080	0.080	0.080	0.080	0.080	0.058	0.060	0.069	0.008							
T. atroviride voucher TriAtv JSB123 (KC569357.1)	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.085	0.091	0.094	0.085	0.087						
T. viride strain ATCC 28038 (AY380909.1)	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.084	0.091	0.094	0.085	0.087	0.004					
T. vinosum strain GJS 99-158 (AY380904.1)	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.084	0.091	0.094	0.085	0.087	0.004	0.004				
T. gracile strain GJS 10-263 (JN175488.1)	1.546	1.546	1.546	1.546	1.546	1.546	1.546	1.402	1.434	1.447	1.535	1.578	1.521	1.562	1.562			
T. harzianum voucher TriH JSB971 (KC569354.1)	0.082	0.082	0.082	0.082	0.082	0.082	0.082	0.060	0.062	0.071	0.006	0.002	0.089	0.089	0.089	1.568		
T. harzianum voucher 60693 (KC569343.1)	0.087	0.087	0.087	0.087	0.087	0.087	0.087	0.064	0.066	0.075	0.014	0.010	0.094	0.094	0.094	1.585	0.008	
T. virens strain PUXX-FS22 (KR296891.1)	0.080	0.080	0.080	0.080	0.080	0.080	0.080	0.055	0.057	0.066	0.019	0.017	0.087	0.087	0.087	1.566	0.014	0.023

Rhizoctonia solani has been identified able to cause disease in lower steam and roots of plants. *Rhizoctonia solani* is the important pathogens for maize, rice, wheat, cotton, and other important crops, including vegetables [19]. Some fungi such as *Trichoderma* has been identified able to inhibits *Rhizoctonia solani* grows. Scholars point out that there are variation in ability and affectivity of *Trichoderma* to inhibits *Rhizoctonia solani*. *Trichoderma asperellum* able to inhibits *Rhizoctonia solani* about 74.4%. This inhibition was higher than inhibition of *Trichoderma harzianum* and *Tricoderma* spp.

The ability of *Trichoderma* to produce lytic enzymes such as cellulose, chitinase, glucanase and protease, especially in *Trichoderma asperellum* depend on the carbon sources [20]. In the field, *Trichoderma asperellum* has ability to inhibits more than 80% of *Rhizoctonia solani,* compared to *Trichoderma* spp. isolates. This data shows that basically soil of South Kalimantan rich in term of *Trichoderma*. The management of soil is especially important to conserve *Trichoderma*. Forest fires an important aspect in land degradation in South Kalimantan. In such a case forest and shrubs lands should be minimized. Fundamentally, soil conservation is important to maintain soil biodiversity, including genetic diversity levels of soils biota [21] [22].

IV. Conclusion

The sample of *Trichoderma* with codes BM (Banjarmasin), KB (Kota Baru), TB (Tanah Bumbu), B (Banjar), BK (Barito Kuala) and TL (Tanah Laut) has similarity about 100 % with *Trichoderma asperellum strain ZWPBG1* (KR868286.1). This sample also has 98.76% similarity with *Trichoderma atroviride* voucher TriAtv JSB123 (KC569357.1), *Trichoderma viride* strain ATCC 28038 (AY380909.1) and *Trichoderma Vinosum* strain GJS 99-158 (AY380904.1). The sample similarity with *Trichoderma harzianum* voucher 60693 (KC569343.1), *Trichoderma harzianum voucher TriH JSB301* (KC569359.1), *Trichoderma harzianum* TriH JSB971 (KC569354.1) was about 91.75%. Compared to the *Trichoderma virens strain* PUXX-FS22 (KR296891.1), samples has similarity about 91.99%

References

[1]. S. Savary, L. Willocquet, FA. Elazegui, NP. Castilla and PS. Teng, Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations, Plant disease, 84(3), 2000, 357-369.
[2]. GE. Harman, CR. HowellA. Viterbo, I. Chet and M. Lorito, Trichoderma species—opportunistic, avirulent plant symbionts. Nature reviews microbiology, 2(1), 2004, 43-56.
[3]. JR. Parmeter, Rhizoctonia solani, biology and pathology (Univ of California Press, 1970).
[4]. Y. Elad, I. Chet and J Katan, Trichoderma harzianum: A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani. Phytopathology, 70(2), 1980, 119-121.
[5]. IR. Sanders, JP. Clapp and A. Wiemken, The genetic diversity of arbuscular mycorrhizal fungi in natural ecosystems–a key to understanding the ecology and functioning of the mycorrhizal symbiosis. New Phytologist, 133(1), 1996, 123-134.
[6]. SB. Brush, Genes in the field: on-farm conservation of crop diversity (IDRC, 2000).
[7]. DL. Hawksworth, Fungal diversity and its implications for genetic resource collections. Stud Mycol, 50, 20049-18.
[8]. M. Wuczkowski, I. Druzhinina, Y. Gherbawy, B. Klug, H. Prillinger and CP. Kubicek, Species pattern and genetic diversity of Trichoderma in a mid-European, primeval floodplain-forest. Microbiological research, 158(2), 2003, 125-133.
[9]. I. Gomez, I Chet and A. Herrera-Estrella, Genetic diversity and vegetative compatibility among Trichoderma harzianum isolates. Molecular and General Genetics MGG, 256(2), 1997, 127-135.
[10]. MR. Hermosa, E. Keck, I. Chamorro, B. Rubio, L. Sanz, JA. Vizcaíno and E. Monte, Genetic diversity shown in Trichoderma biocontrol isolates. Mycological research, 108(8), 2004, 897-906.
[11]. IS. Druzhinina, AG. Kopchinskiy, CP. Kubicek, The first 100 Trichoderma species characterized by molecular data. Mycoscience, 47(2), 2006, 55-64.
[12]. J. Doyle, DNA protocols for plants. In Molecular techniques in taxonomy (pp. 283-293) (Springer Berlin Heidelberg. 1991).
[13]. CL. Schoch, KA. Seifert, S. Huhndorf, V. Robert, JL. Spouge, CA. Levesque and AM. Miller Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences, 109(16), 2012, 6241-6246.
[14]. RH. Nilsson, M. Ryberg, E. Kristiansson, K. Abarenkov, KH. Larsson and U. Kõljalg, Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS One, 1(1), 2006, e59.
[15]. GC. Samuels, A. Ismaiel, MCC. Bon, S. De Respinis, and O. Petrini, Trichoderma asperellum sensu lato consists of two cryptic species. Mycologia, 102(4), 2010, 944-966.
[16]. CP. Kubicek and GE. Harman, Trichoderma and Gliocladium. Volume 1: Basic biology, taxonomy and genetics (Taylor & Francis Ltd. 1998).
[17]. J. Bissett, Trichoderma atroviride. Canadian Journal of Botany, 70(3), 1992, 639-641.
[18]. M. Abdollahi, K. Rahnama, M. Marabadi, F. Ommati, M. Zaker, The in vitro efficacy of Trichoderma isolates against Pythium aphanidermatum, the causal agent of sugar beet root rot. Journal of Research in Agricultural Science, 8(1), 2012, 79-87.
[19]. JR. Parmeter, Rhizoctonia solani, biology and pathology (Univ of California Press, 1970)
[20]. S. de los Santos-Villalobos, DA. Guzmán-Ortiz, MA., Gómez-Lim, JP. Délano-Frier, S. de-Folter, P. Sánchez-GarcíaJJ. Peña-Cabriales, Potential use of Trichoderma asperellum (Samuels, Liechfeldt et Nirenberg) T8a as a biological control agent against anthracnose in mango (Mangifera indica L.). Biological Control, 64(1), 2013, 37-44.
[21]. DH. Reed and R. Frankham, Correlation between fitness and genetic diversity. Conservation biology, 17(1), 2003, 230-237.
[22]. SB. Brush, Genes in the field: on-farm conservation of crop diversity (IDRC, 2000).

INVESTIGATION OF B. BASSIANA PERSISTENCE AND VIRULENCE FACTOR AGAINST P. XYLOSTELLA OF PEATLAND IN CENTRAL KALIMANTAN

ORIGINALITY REPORT

14%	8%	9%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

3%

★ M. Chaithra, N. S. Pankaja, P. S. Benherlal, H. B. Mahesh. " Micro-farming specific morpho-molecular characterization of isolates and its bio-control potential against foliar fungal plant pathogens ", Archives of Phytopathology and Plant Protection, 2023

Publication

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		