Antagonistic Activity against Plant Pathogenic Fungus by Various Indigenous Microorganisms from Different Cropping Systems in Soc Trang Province, Vietnam

Le Thi Xa¹ and Nguyen Khoi Nghia²*

¹Biotechnology Research and Development Institute, Can Tho University, Can Tho City, Vietnam
²Department of Soil Science, College of Agriculture and Applied Biology, Can Tho University, Can Tho City, Vietnam

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ABSTRACT

This study assessed antagonistic capacity of various indigenous microorganisms (IMO) collected from different cropping systems within Soc Trang province against plant pathogenic fungus including Fusarium oxysporum and Rhizoctonia solani. Biocontrol activity of fifteen collected IMOs was investigated on PDA agar media for 5-7 days under laboratory conditions with three different scenarios. IMO and pathogenic fungus were incubated at the same time and IMO was introduced before and after inoculation of plant pathogenic fungus. The results illustrated that all surveyed IMOs were found to have highly potential biocontrol against two plant pathogenic fungi to different extents and IMOs which were introduced before the inoculation of pathogenic fungi showed the highest efficiency in biocontrol of plant pathogen. Particularly, four out of fifteen IMOs which were collected from bamboo, shallot, grapefruit and guava farms showed their highest antagonistic efficacy on Fusarium oxysporum biocontrol by completely decaying fungal hyphae of this fungal strain after seven incubation days. For Rhizoctonia solani, all IMOs displayed highly antagonistic ability with inhibitory percentages varying between 52.96% and 92.59% after two days. The antagonistic functions of all collected IMOs could be exploited for plant protection from plant-pathogenic fungus.

Keywords: Antagonism/ Fungal plant pathogens/ Fusarium oxysporum/ Indigenous microorganism/ Rhizoctonia solani

* Corresponding author:
E-mail: nknghia@ctu.edu.vn

1. INTRODUCTION

The concept of indigenous microorganisms (IMO) was developed by Kyu in the 1960s at the Janong Farming Institute, South Korea. IMO-based technology is a great technology applied in the eastern part of the world. IMO cultures contain consortia of beneficial microorganisms comprising of fungi and bacteria that are deliberately collected and cultured from soils to enhance plant growth promotion (Reddy, 2011). Indigenous micro-organisms are a group of innate microbial consortium that inhabit the soil and the surfaces of all living things. It has a potential in biodegradation, bioleaching, bio-composting, nitrogen fixation, phosphate solubilization, soil fertility improvement and in the production of plant growth hormones as well as biocontrol (Kumar and Gopal, 2015). In fact, according to Chiemela et al. (2013b), many studies indicated that application of IMO in agriculture has been a friendly environmental method and helped to enhance organic matter decomposition, plant nutrition, soil fertility, crop yields and resistance to plant diseases. Application of IMO is effective in compost production since it promotes the rapid degradation of agricultural and plant residues, producing large amounts of micronutrients in the soluble form that are very easy to be taken up by plants (Chiemela et al., 2013a). Another research study was conducted in Hawaii to evaluate the biocontrol function of IMOs against Ceratocystis sp. fungus causing a deadly plant disease for Ohia trees. The result showed that IMOs had a very good function in recovery of Ohia trees’s health from plant disease (Board of Land and Natural Resources State of Hawaii, 2018). Moreover, we know that different cropping system and management practices play an important role in soil physico-chemical properties and microbiome composition and diversity (Fierer et al., 2012; Zhang et al., 2016). Thus, in this study we sampled many different locations corresponding to different cropping systems and
management practices with an aim of having more choices in finding the very best microbial community in biocontrol for some very common plant pathogens, *Fusarium oxysporum* and *Rhizoctonia solani*. These pathogens cause many diseases for many kinds of economic crop plants in the Mekong Delta Region of Vietnam where agriculture is very important. However, deep and scientific knowledge about the abilities of IMO for biocontrol of plant pathogens are still lacking and should be scientifically elucidated. Therefore, the aim of this study was to assess antagonistic activity against plant pathogenic fungus of various indigenous microorganism communities from different agro-ecosystems.

2. METHODOLOGY

2.1 IMO sample collection

Fourteen different IMOs were collected from different crop models in Soc Trang Province, Vietnam including bamboo, crop rotation (corn, watermelon, courgette), banana, shallot, vegetables, rice, watermelon, grassland, maize, lettuce, oranges, grapefruit, guava, and sugarcane by following the method described by Kyu and Koyama (1997) (Figure 1).

![IMO Procedure](image)

**Figure 1.** Procedure of IMO collection

Sites of sample collection are presented in Table 1. At each sampling site, three plastic baskets (25 × 15 × 8 cm) were used, corresponding to 3 replicates. Each basket was filled with 1 kg of steamed rice and covered using cloth and a waist belt. The baskets were buried under ground at a depth of 20-30 cm at each sampling site and covered with leaf litter for three days. After four days of incubation, the fermented rice samples colonized by indigenous microorganisms were harvested. The microorganism-infected rice were put into a glass jar and carried to the laboratory. This source of microorganisms was called IMO₁. Mix IMO₁ was prepared by combining an equal amount of 150 g of each IMO₁ together. All collected IMO₁ were mixed well with brown sugar with a ratio of 1:1 (w/w) until the mixed material became gooey. These mixed materials were stored in ceramic pots in a cool area and away from direct sunlight for seven days for another fermentation time. After seven days of fermentation, these sources of microorganisms were called IMO₂. The IMO₂ were kept in the refrigerator at 4 °C for further studies.

| Code | Origin of IMO | Located in Soc Trang Province          |
|------|---------------|----------------------------------------|
| IMO1 | Bamboo        | Phu Tam Commune, Chau Thanh District   |
| IMO2 | Crop rotation | 5 Ward, Soc Trang City                 |
| IMO3 | Banana        | 7 Ward, Soc Trang City                 |
| IMO4 | Shallot       | Vinh Phuoc Commune, Vinh Chau District |
| IMO5 | Lettuce       | 3 Ward, Soc Trang City                 |
| IMO6 | Rice          | My Xuyen Town, My Xuyen District        |
Table 1. The location of fourteen IMO samples in Soc Trang Province (cont.)

| Code | Origin of IMO | Located in Soc Trang Province                  |
|------|---------------|-----------------------------------------------|
| IMO7 | Watermelon    | Truong Khanh Commune, Long Phu District       |
| IMO8 | Grassland     | Truong Khanh Commune, Long Phu District       |
| IMO9 | Maize         | Thanh Tri Commune, Thanh Tri District         |
| IMO10| Vegetables    | Thanh Quoi Commune, My Xuyen District         |
| IMO11| Oranges       | Xuan Ho Commune, Ke Sach District             |
| IMO12| Grapefruit    | Xuan Ho Commune, Ke Sach District             |
| IMO13| Guava         | Xuan Ho Commune, Ke Sach District             |
| IMO14| Sugarcane     | Dai An II Commune, Cu Lao Dung District       |

2.2 Plant pathogenic fungal sources

The two fungal pathogens used in this study were *Fusarium oxysporum* and *Rhizoctonia solani*. The first one causes wilt disease on sesame and the latter causes a root rot disease on vegetables. These fungal sources were provided from the plant pathogen laboratory, Plant Protection Department, College of Agriculture, Can Tho University.

2.3 Evaluation of IMO with antagonistic potentials

An aliquot of 10 g of each IMO was transferred into 250 mL glass bottles containing 90 mL sterilized distilled water. The bottles were put on a horizontal shaker at a speed of 150 rpm for 60 min, and then left to stand for 10 min. This microbial IMO suspension was used as a microbial source for antagonistic experiments.

For the fungus, *Fusarium oxysporum*, biological control activity of fifteen collected IMO was investigated on PDA agar media with three different scenarios: (1) IMO and *Fusarium oxysporum* were incubated on PDA agar media at the same time and with a 4 cm interval space between the two colonies; (2) IMO were introduced two days before *Fusarium oxysporum* inoculation; and (3) IMO were introduced two days after *Fusarium oxysporum* inoculation.

Unlike *Fusarium oxysporum*, the growth of fungus *Rhizoctonia solani* was very fast and after just two days of incubation, the fungal hyphae of *Rhizoctonia solani* was spread fully on the PDA medium. Therefore, for the case of *Rhizoctonia solani* only two scenarios were applied: (1) IMO and *Rhizoctonia solani* were introduced on PDA agar media at the same time and with a 4 cm interval space between two colonies; and (2) IMO was introduced two days after *Rhizoctonia solani* inoculation.

Antagonistic activity of IMOs against fungi *Fusarium oxysporum* and *Rhizoctonia solani* was assessed by bilateral symmetrical implantation technique adopted by Vincent (1947). A brief description is described as follows: A five millimeter diameter core of PDA agar containing five days old culture of targeted fungi was taken and inoculated into the center of the right half of a Petri dish in complete aseptic condition. Then a 20 µL aliquot of IMO suspension was transferred into the center of the left half of the left Petri dish in complete aseptic condition and left for 30 min to dry completely. The PDA media containing Petri dish without IMO inoculation served as a negative control. Each IMO was tested in three replicates. All the Petri dishes were incubated at room temperature and dark condition during the incubation period. Radical growth of colonies was measured after seven days of incubation for *Fusarium oxysporum* and after five days of incubation for *Rhizoctinia solani*. The result of mycelia growth was expressed as the mean of the triplicates. Percent inhibition of mycelia growth over control was calculated by the formula given by Vincent (1947).

\[
H(\%) = \left(\frac{D_c - D_t}{D_c}\right) \times 100
\]

Where; H: inhibition efficacy (%)  
Dc: diameter of mycelia growth in control  
Dt: diameter of mycelia in treated

2.4 Data analysis

The data were analyzed by ANOVA and compared by DUNCAN test with MINITAB software with 16.2 version.

3. RESULTS AND DISCUSSION

3.1 Antagonistic potentials of IMOs with *Fusarium oxysporum*

The results of the study of the antagonistic activity of 14 different IMOs from different cropping systems and mixed IMO against fungus *F. oxysporum*,
presented in Table 2 and Figure 2, indicated that all 15 tested IMOs had a highly antagonistic effect against *F. oxysporum* with significantly different extents. Among three scenarios, it can be seen clearly from Table 2 that the percentage of mycelia inhibition were recorded as follows: IMO were introduced on PDA containing Petri dish two days before fungus *F. oxysporum* > IMO and fungus *F. oxysporum* were introduced on PDA containing Petri dish at the same time > IMO were introduced on PDA containing Petri dish two days after fungus *F. oxysporum*.

For the case that IMOs were incubated two days before fungus *F. oxysporum* inoculated, the mycelia inhibition of all 15 tested IMOs were recorded to be very high with the amount varying between 44.98% and 100%. The highest antagonistic effects of IMO against fungus *F. oxysporum* were determined for four containing Petri dish two days before fungus *F. oxysporum* > IMO and fungus *F. oxysporum* were introduced on PDA containing Petri dish at the same time > IMO were introduced on PDA containing Petri dish two days after fungus *F. oxysporum*.

**Table 2.** Mycelia inhibitory capacity of 15 IMOs for fungus *Fusarium oxysporum* after 7 days of incubation

| Code  | Origin of samples | Inhibition efficacy (%) |
|-------|-------------------|-------------------------|
|       | IMO>Fungus         | IMO= Fungus             | IMO<Fungus | Average |
| IMO1  | Bamboo            | 100.0\textsuperscript{a} | 100.0\textsuperscript{a} | 25.25\textsuperscript{cd} | 75.08 |
| IMO2  | Crop rotation     | 80.14\textsuperscript{b} | 54.08\textsuperscript{cd} | 25.57\textsuperscript{cd} | 52.26 |
| IMO3  | Banana            | 81.19\textsuperscript{b} | 44.90\textsuperscript{ef} | 22.95\textsuperscript{cd} | 49.68 |
| IMO4  | Shallot           | 100.0\textsuperscript{a} | 47.48\textsuperscript{c}  | 23.78\textsuperscript{cd} | 57.08 |
| IMO5  | Lettuce           | 68.99\textsuperscript{bcd} | 49.66\textsuperscript{de} | 24.26\textsuperscript{cd} | 47.64 |
| IMO6  | Rice              | 66.20\textsuperscript{de} | 67.01\textsuperscript{b}  | 40.98\textsuperscript{bc} | 58.06 |
| IMO7  | Watermelon        | 44.98\textsuperscript{f} | 45.24\textsuperscript{ef} | 22.30\textsuperscript{cd} | 37.50 |
| IMO8  | Grassland         | 51.97\textsuperscript{g} | 38.79\textsuperscript{f}  | 13.12\textsuperscript{d}  | 34.63 |
| IMO9  | Maize             | 60.98\textsuperscript{def} | 51.70\textsuperscript{de} | 26.56\textsuperscript{c}  | 46.41 |
| IMO10 | Vegetables        | 70.73\textsuperscript{bcd} | 52.04\textsuperscript{de} | 48.53\textsuperscript{bc} | 57.10 |
| IMO11 | Oranges           | 58.54\textsuperscript{def} | 38.10\textsuperscript{f}  | 26.56\textsuperscript{c}  | 40.07 |
| IMO12 | Grapefruit        | 100.0\textsuperscript{a} | 100.0\textsuperscript{a}  | 46.89\textsuperscript{bc} | 82.30 |
| IMO13 | Guava             | 100.0\textsuperscript{a} | 100.0\textsuperscript{a}  | 56.39\textsuperscript{a}  | 85.46 |
| IMO14 | Sugarcane         | 53.31\textsuperscript{ef} | 46.60\textsuperscript{e}  | 26.23\textsuperscript{c}  | 42.05 |
| Mix IMO | Mixed          | 76.31\textsuperscript{bc} | 54.42\textsuperscript{cd} | 26.87\textsuperscript{b}  | 52.53 |

Note: Values in the same column with the same letters are not significant difference (p<0.05); “=” means IMO and *F. oxysporum* were incubated at the same time; “>” means IMO was introduced 2 days before *F. oxysporum* and “<” means IMO was introduced 2 days after *F. oxysporum*.  

**Figure 2.** Antagonistic activities of 15 IMOs against fungus *F. oxysporum* after 7 days of incubation in the case IMOs introduced 2 days after *F. oxysporum*; “F”: *F. oxysporum* and “I”: IMO.
IMOs from bamboo, shallot, grapefruit, and guava farms with the mycelia inhibition of up to 100% and also significantly higher than those of the remaining IMOs (p<0.05), since these four IMOs decayed completely fungal hyphae of fungus *F. oxysporum*. In addition, two IMOs collected originally from crop rotation and banana cultivation fields had the ability to stop the growth of *F. oxysporum* mycelia of up to 80% even when IMOs were incubated on PDA agar containing Petri dish two days before fungus *F. oxysporum*. Also in this scenario, the remaining IMOs showed good resistance against *F. oxysporum*, with antagonistic efficacy ranged from 51.97% to 73.31%, except for IMO of grassland which had an antagonistic efficacy of 44.98%.

Regarding the scenarios that both IMO and fungus *F. oxysporum* introduced on PDA containing Petri dish at the same time, there was a slight decrease in inhibition efficacy of some IMOs when compared to the case of IMO was incubated two days before fungus. However, the mycelia inhibition of three tested IMOs from bamboo, grapefruit, and guava were still very high with the stable amount at 100%, while the inhibition of mycelia dropped to 47.38% for IMO from shallot farm. In addition, the IMOs collected from rice, watermelon resisted stably against *F. oxysporum* in both cases and with the mycelia inhibition of around 66% and 45%, respectively. Particularly, IMOs collected from grassland and orange farms had the lowest antagonistic effect against *F. oxysporum* and with the inhibition efficacy was just over 38% whilst the seven remaining tested IMOs including crop rotation, banana, maize, lettuce, vegetables, sugarcane and mixture had a slight drop of inhibition efficacy and this value varied between 44.90% and 54.42%.

For the case that IMOs were incubated two days after fungus *F. oxysporum* incubation. It was found that the inhibitory effect of all fifteen IMO were lowest. The mycelia inhibition of all 15 tested IMOs showed a significant inhibition with a rate varied between 13.12% and 56.39% and also had a slight decrease in inhibition efficacy of some IMOs as compared with two other scenarios. The highest antagonistic effects of IMO against fungus *F. oxysporum* were determined for IMOs collected from guava farm and significantly higher than others. In addition, three IMOs collected from rice, vegetables and grapefruit farms had a high inhibitory effect on the growth of *F. oxysporum* mycelia with the inhibition efficacy was found at 40.98%, 48.53%, and 46.98%, respectively. Meanwhile, the mycelia inhibition of the IMOs from bamboo, crop rotation, banana, shallot, watermelon, maize, lettuce, oranges, sugarcane, and mixture varied between 22.3% and 26.87%. Particularly, IMO collected from grassland had the lowest antagonistic effect against *F. oxysporum* (13.12%). It means that this IMO had less antagonistic effect against fungus *F. oxysporum* than other IMOs under the condition of *F. oxysporum* introduced two days before.

From the results it can be seen that all surveyed IMOs were found to have great potential in biocontrol against *F. oxysporum*, and IMOs collected from guava and grapefruit cultivation fields showed the highest efficiency as compared to others. They were possible to prevent the growth of *F. oxysporum* up to around 50% even when incubated two days after pathogen fungus and decayed completely fungal hyphae of fungus when introduced two days before fungus (average efficiency was over 80%). Other remaining IMOs had an average efficiency for fungus *F. oxysporum* ranged from 34.63 to 75.08%. This could be explained that microbial community in IMO including fungi, bacteria and actinomycetes could excrete some antimicrobial-like compounds and enzymes to inhibit the growth of mycelium *F. oxysporum* (Xa and Nghia, 2019).

Stanojkovic-Sebic et al. (2017) indicated that it is very hard to completely control the soil-borne diseases caused by *Fusarium* species in soil by means of chemical approaches. Inconsistencies of inhibition effect in biocontrol of soil-borne pathogens under varying environmental conditions were identified as a common limiting factor. However, research focusing on the efficacy of indigenous microbial communities against these soil-borne pathogens is still limited. Huy et al. (2017) recommended that treating seeds with *Trichoderma asperrellum* in a combination with bio-fertilizer about 2-3 days before planting would help plants to develop well, prevent plant diseases and give high biological control efficiency for plant pathogen. The reason for this result was that microorganisms could excrete many enzymes that could destroy the walls of mycelium of fungi and they may also directly kill fungal pathogens.

In general, application of IMOs as a source of beneficial microbes for crops and plants was found to have great inhibitory capacity to *F. oxysporum* with fungal hyphae suppression rates ranged between 34.63% and 85.46%. Additionally, Yuliar et al. (2013) indicated that when 100 µL endophytic bacteria were
used to biocontrol *F. oxysporum*, the mycelia growth inhibition effect was recorded to be up to of 35% after 5 days of incubation. Besides, Dar et al. (2013) investigated the antagonistic potential of some beneficial fungi to *F. oxysporum*, suggesting that they could excrete anti-fungal compounds which may suppress the growth of *F. oxysporum* from 54.4% to 92.5%. A study of Stanojkovic-Sebic et al. (2017) indicated that *Pseudomonas* strains from star anise plantation soil had a great ability to inhibit *F. oxysporum* up to of 77.8%. Similarly, Toppo and Naik (2015) determined the biocontrol activity of nine bacterial strains against *Fusarium* spp. The results showed that these strains had high mycelia growth inhibition capacity and the inhibiting values varied between 33% and 73%.

### 3.2 Antagonistic potentials of IMOs with *Rhizoctonia solani*

The results of antagonistic potentials of 14 different IMOs from different cropping systems and mixed IMO with fungus *Rhizoctonia solani* are presented in Figure 3 and Figure 4. In the case of which both IMOs and fungus were incubated at the same time, IMOs showed less effect in inhibiting of fungus *R. solani* than the case of which IMOs were introduced two days before fungus *R. solani* inoculated and it could be due to the fungal hyphae developed very rapid and it took only two days for fungal hyphae to grow fully on the surface of the containing PDA medium Petri dish dishes (Figure 3). Nevertheless, fungal hyphae of *R. solani* could not touch the growing zones of IMOs, except for the case of IMOs collected from grassland and oranges. This means that these two IMOs did not have an ability to resist fungus *R. solani*. This result was similar to the study of Ghai et al. (2007) who examined the biocompatibility of isolated strains and showed that bacterial isolates strongly resisted against *F. sclerotium*, but did not inhibit effectively fungus *R. solani*.

![Image](image_url)

**Figure 3.** Antagonistic activities of some representative IMOs against fungus *Rhizoctonia solani* after 2 days of incubation (Note: A: IMO and *R. solani* were incubated at the same time; B: IMO was incubated 2 days before *R. solani*; “R” means Rhizoctonia solani; “I” means IMO)

When IMOs were placed on PDA agar medium two days before fungus inoculation, the high anti-fungal capacities of IMOs were recorded with a large variation. The mycelial growth of fungus *R. solani* was inhibited by IOMs with a range varying between 52.96% and 92.59%. It can be seen that maximum inhibition capacity was observed for IMO collected from guava cultivation farm with the rate of efficiency over 90%, closely followed by IMO from grapefruit farm (88.19%). The lowest inhibition efficacy to fungus *R. solani* were found between 52.96% and 54.26% for grassland and maize IMOs while others showed inhibition rates between 59.26% and 71.11%.
From this result, it can be seen that all 15 tested IMOs had very good function in antagonisms against fungal pathogen *F. oxysporum* better than against fungus *Rhizoctonia*, the inhibitory effect of IMOs for both two fungi was shown to be more effective when IMOs were placed before fungi (from 50% to 93% of inhibitory efficacy). This implies that IMO can be used to prevent the soil born diseases like *Fusarium oxysporum* and *Rhizoctonia solani*. The result of this study was consistent with study of Ghai et al. (2007) and Robles-Yerena et al. (2010). Their results showed that when *Ascomycete* and plant pathogenic fungus *P. capsici* were introduced at the same time, about 53.1% of *P. capsici*’s milleial growth was inhibited. When *Ascomycete* was incubated 3 days before fungus *P. capsici*, the inhibition capacity toward the growth of micellia was improved up to of 73%. However, *Ascomycete* did not have any effect on fungus *Rhizoctonia solani*. Similarly, Ramzan et al. (2014) obtained 15 indigenous bacteria and 44 fungi and tested for their inhibition effect on soil-borne pathogens such as *Fusarium solani*, *Macrohomina phaseolina*, *Pythium aphanidermatum*, *Rhizoctonia solani*, and *Sclerotium rolfsii* under in vitro conditions. The result indicated that a total of 20 fungal and 7 bacterial strains showed their high biocontrol efficiency in dual culture plate assay against these soil-borne pathogens. Furthermore, Koch et al. (2013) found that the antifungal compounds extracted from culture solution were found to be very effective in inhibiting the growth of fungus up to of 42.79% and 20.45% for *Rhizoctonia solani* and *Fusarium solani*, respectively. Moreover, according to Duffy and Weller (1995) and Hervas et al. (1998), a mixture of compatible biocontrol agents was an ecologically sound approach to biocontrol of soil borne diseases, especially when used in combination with limited or partial resistance. Mixtures of different species of microorganisms may result in better plant colonization, may be better adapted to environmental changes, may present a large number of pathogen suppressive mechanisms, and/or may protect against a broad range of pathogens. In short, all achieved results of this study and some other previous studies’ results supported our finding about the biocontrol function of IMOs against plant pathogenic fungi such as *F. oxysporum* and *R. solani*. However, the mechanisms in biocontrol function of IMOs should be intensively investigated more in further studies.

4. CONCLUSION

Biological control of phytopathogenic fungi is an ecological approach of plant protection. This investigation confirmed highly pronounced antifungal activity of all tested IMOs collected from different farming system habitats within Soc Trang Province, Vietnam. With IMO, there is no need to isolate single
strains of microorganisms for application purposes, while still having good function in anti-fungal capacity to a high extent beside other functions like plant growth promotion which has been proven via previous studies. Two IMOs collected from guava and grapefruit farms showed their most pronounced activity in biological control of two plant pathogenic fungi Fusarium oxysporum and Rhizoctonia solani. The results of this study imply that the indigenous microorganism communities have a great potential in biological control of phytopathogen fungi in soil and can be exploited in plant protection.

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