

Title	Antagonistic Activity of <i>Trichoderma</i> Strains Against Pathogenic Fungi of <i>Arachis hypogaea</i> L.
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Citation	
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Antagonistic Activity of *Trichoderma* Strains Against Pathogenic Fungi of *Arachis hypogaea* L.

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Abstract

Fungi were isolated from the rhizospheric soils and infected leaves of *Arachis hypogaea* L. (groundnut) collected from Shwe Kyethtau village, Sagaing Township, Sagaing Region. The fungi were isolated from rhizospheric soil using the Rose Bengal Medium for (KYZN 01), the Potato Dextrose Agar (PDA) medium for (KYZN 02) and (KYZN 03), from the infected leaves using the PDA medium. Pathogenic fungi were isolated from the infected parts of groundnut such as root rot, crown rot and pod rot by using PDA medium. The resulting pathogenic fungi were confirmed (KYZN 04) as *Macrophomina* sp., (KYZN 05) as *Aspergillus* sp. and (KYZN 06) as *Fusarium* sp.. The assay for antagonism was performed on PDA medium by dual culture method. The maximum inhibitory activity of *Trichoderma* strain (KYZN 01) was 69% against *Macrophomina* sp. and followed by 66% on *Fusarium* and 50% on *Aspergillus* sp.. *Trichoderma* strain (KYZN 02) showed maximum inhibition against 61% on *Macrophomina* sp. and followed by 50% on *Aspergillus* sp. and 48% on *Fusarium* sp. *Trichoderma* strain (KYZN 03) showed the highest effects 72% against *Macrophomina* sp. but minimum inhibition was in 60% on *Fusarium* sp. and 56% on *Aspergillus* sp.. The significant inhibition of all three strains were observed in *Macrophomina* sp.. KYZN 03 was maximum inhibition percentage against on the all pathogenic fungi. All of the *Trichoderma* strains have significantly inhibition on pathogenic fungi which induce the major diseases symptom of groundnut plants. *Trichoderma* strains can be used as effective biocontrol agents for the diseases of groundnut plants.

Keywords: Antagonistic, *Trichoderma* strains, pathogenic fungi

Introduction

Groundnut plant is a legume crop that belongs to the family Fabaceae, genus *Arachis*, and botanically named as *Arachis hypogaea* L. It is one of the world's most popular oil seed crops. Its high content of oil and protein makes it an important commodity for both human use and livestock feed (Farak & Zahran 2014).

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The largest oilseed crop production area was Mandalay and Magway Divisions, with 149, 639 ha and 120, 477 ha in rainy seasons (Soe Soe Win 2007). Many plant species have been destroyed by plant pathogens with strongly damaged the crop yield. The fungus infects lower stems of groundnut, which are in contact with the soil as well as pegs, pods and roots (Adhilakshmi *et al.* 2013). Soil texture affected incidence of root and pod rot of groundnut caused by several fungi i.e. *Fusarium* sp., *Macrophomina* sp., *Rhizoctonia* sp. and *Aspergillus* sp. (Faujdar & Oswalt 1992).

Fungicides are widely used for controlling the various disease of plants. Control the diseases using chemical fungicides lead to pollution of atmosphere and have adverse effects on human health. Microorganisms as biocontrol agents have high potential to control plant pathogens and have no negative effect on the environment (Kavitha & Nelson 2013).

Antagonists are biocontrol agents such as bacteria, fungi, actinomycetes, viruses and nematodes that reduce the number of disease producing activities of the pathogens (Killanie *et al.* 2011). Biological control agents include strains belong to fungal genera such as *Trichoderma* sp., *Candida* and *Gliocladium* and bacterial genera such as *Bacillus* and *Pseudomonas*. Among the BCAs, *Trichoderma* sp. are the most intensively studied species (Hui 2013). In this study, *Trichoderma* sp. was isolated from the rhizospheric soil of *Arachis hypogaea* L. *Trichoderma* species are free living fungi that occur in nearly all the soils and other natural habitats. Tran (2010) reported that they are not only parasite of fungal plant pathogens but also can produce antibiotics.

The aim of this research work is to study the macroscopical and microscopical characters of *Trichoderma* strains isolated from rhizospheric soil and leaves of groundnut plants. To achieve this aims, the objectives are to isolate and characterize the pathogenic fungus strains from *Arachis hypogaea* L. and to investigate the antagonistic activity of *Trichoderma* strains against the pathogenic fungi of *Arachis hypogaea* L..

Materials and Methods

Study Area

Soil samples and plant material samples were studied from the groundnut plants growing in agricultural regions of Shwe Kyathtauk village, Sagaing Township, Sagaing Region in July 2016.

Collection of Soil Samples

Five soil samples were collected from the rhizospheric soil of healthy groundnut plants in different site of groundnut field in July 2016 (Attitalla *et al.* 2012).

Isolation of Antagonists

The fungus antagonists were isolated from the rhizosphere soil of groundnut, using serial dilution and pour plate technique on Rose Bengal Medium and Potato Dextrose Agar Medium (Johnson & Curl 1972).

Collection of Pathogenic Plant Samples

Morphological characters of groundnut specimens were studied, then identifications were made using keys and descriptions of Backer and Brink (1965).

Isolation Method for Plant Pathogenic Fungi

Plant pathogenic fungi were isolated by using (Ando & Inada 2004). The identification of *Trichoderma* strains and pathogenic fungi were identified by Barnett (1955) and Bessay (1952).

Growth Inhibition Assay by Dual Culture Method

Interaction between antagonistic efficacy of fungi and pathogenic fungi were determined by the method of Thanh *et al.* 2014.

$$\text{Percent Inhibition of Radial Growth (PIRG)} = A_1 - A_2/A_1 \times 100$$

Where,

A_1 = the radius of pathogenic fungi mycelium in the control plate

A_2 = the radius of pathogenic fungi mycelium in dual culture plate

PIRG = Percent Inhibition of Radial Growth

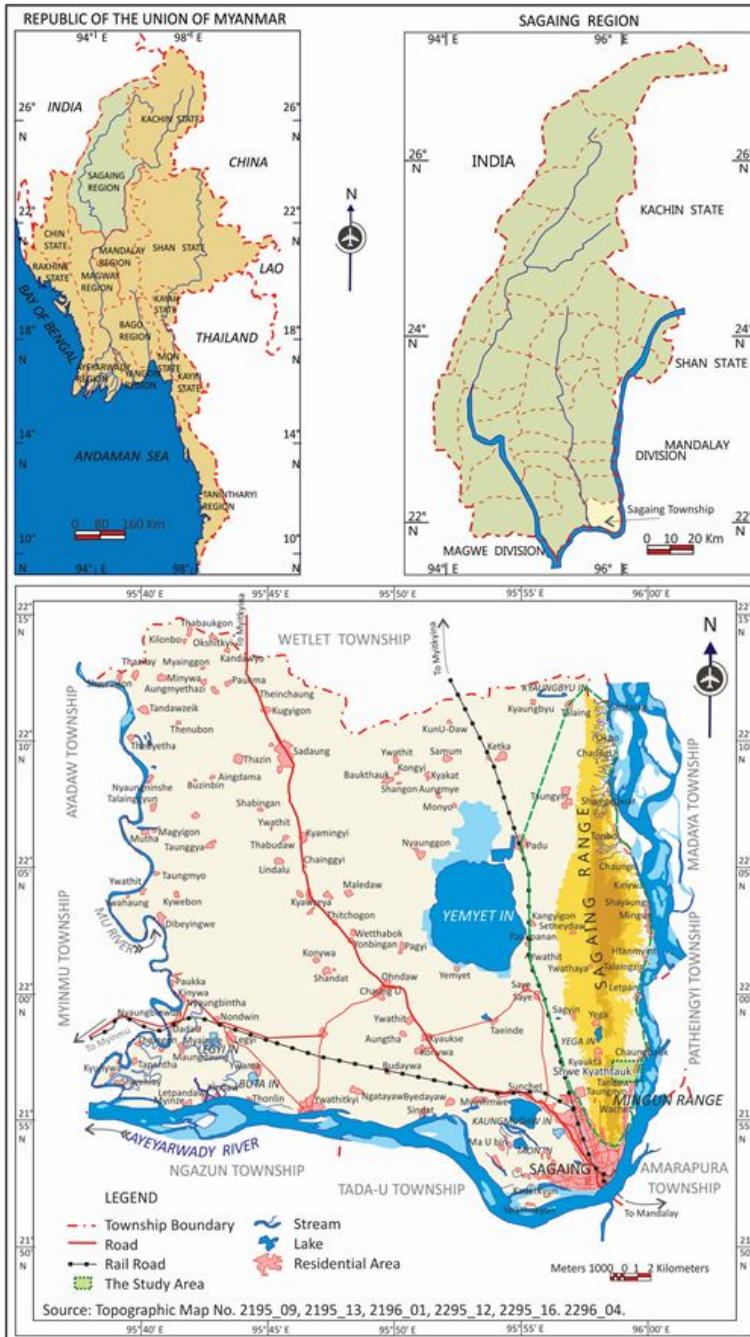


Fig. 1 Location map of specimen Collection Site in Shan Ka Lay Kyun village Amarapura Township

Results

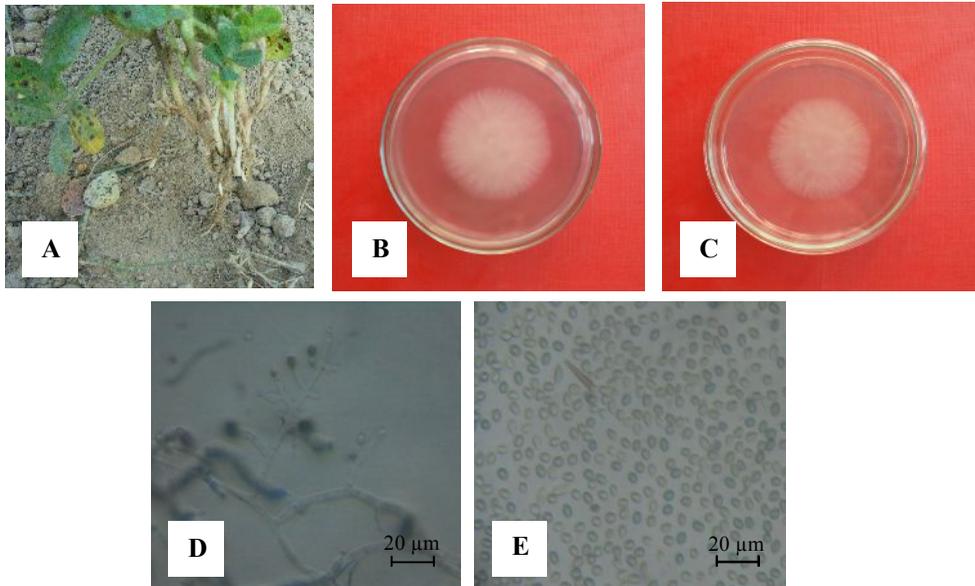


Figure 2. A. *Arachis hypogaea* L. with Rhizospheric soil
 B. Surface colony characters of *Trichoderma* strain (KYZN 01) on PDA medium (3 days)
 C. Reverse colony characters of *Trichoderma* strain (KYZN 01)
 D. Hyphae of *Trichoderma* strain (KYZN 01)
 E. Conidia of *Trichoderma* strain (KYZN 01) (arrow)

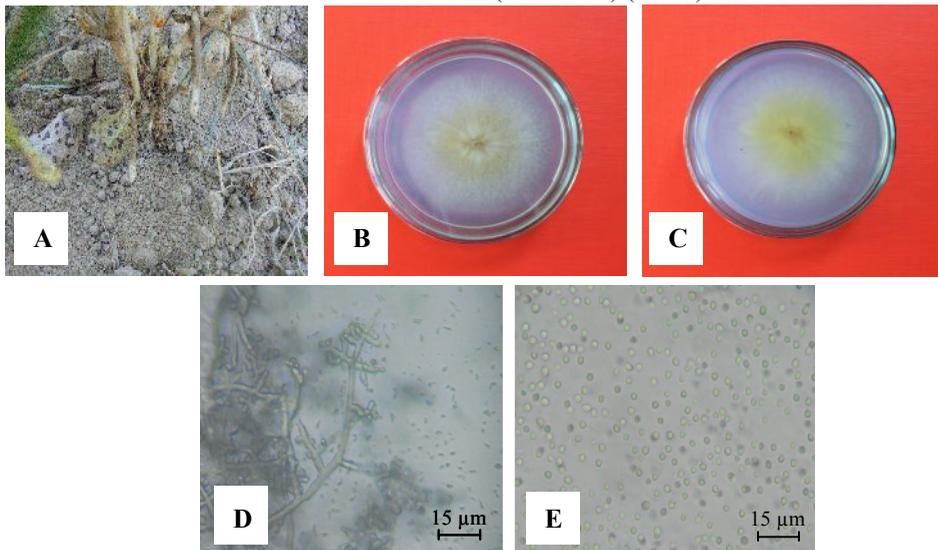


Figure 3. A. *Arachis hypogaea* L. with Rhizospheric soil
 B. Surface colony characters of *Trichoderma* strain (KYZN 01) on PDA medium (3 days)
 C. Reverse colony characters of *Trichoderma* strain (KYZN 01)
 D. Hyphae of *Trichoderma* strain (KYZN 01)
 E. Conidia of *Trichoderma* strain (KYZN 01) (arrow)

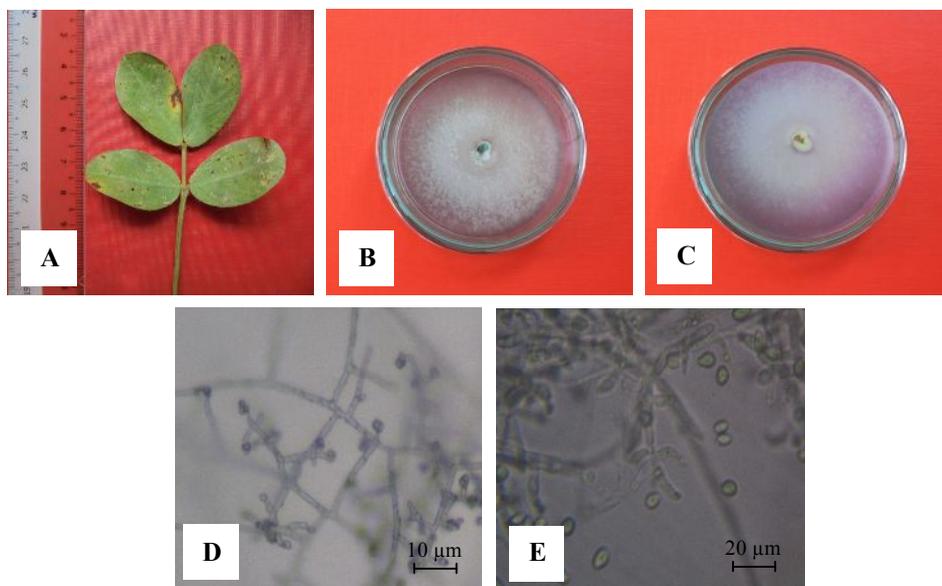


Figure 4. A. Leaf spot disease symptom infected on leaves of groundnut (arrow)
 B. Surface colony characters of *Trichoderma* strain (KYZN 03) on PDA medium (3 days)
 C. Reverse colony characters of *Trichoderma* strain (KYZN 03)
 D. Hyphae of *Trichoderma* strain (KYZN 03)
 E. Conidia of *Trichoderma* strain (KYZN 03) (arrow)

Table (1) Characteristics of isolated antagonists fungi

Isolated strain	Antagonists fungi	Macroscopical characters	Microscopical characters	Sources
KYZN 01	<i>Trichoderma</i> strain	White green, bright green to dull green. Reverse remains white.	Mycelium are septate. Conidiophores upright. Phialides are single, verticillate. Conidia are obvoid.	Rhizospheric soil
KYZN 02	<i>Trichoderma</i> strain	White to greyish and reverse colony in yellowish.	Conidiophores branch. Phialides are in false whorls. Conidia are globose.	Rhizospheric soil
KYZN 03	<i>Trichoderma</i> strain	White to greyish colouration. Reverse remains white.	Mycelium are septate. Conidiophores branch. Phialides are in false whorl. Conidia are short ellipsoid usually rounded.	Leaf spot

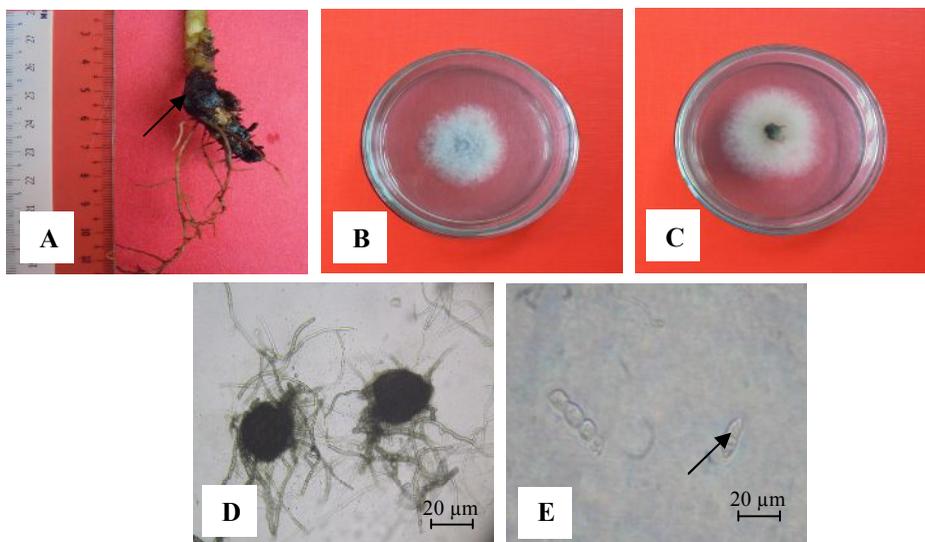


Figure 5. A. Leaf spot disease symptom infected on root of groundnut (arrow)
 B. Surface colony characters of *Macrophomina* sp. (KYZN 04) strain on PDA medium (3 days)
 C. Reverse colony characters of *Macrophomina* sp. (KYZN 04)
 D. Hyphae with pycnidia of *Macrophomina* sp.
 E. Conidia of *Macrophomina* sp. (arrow)

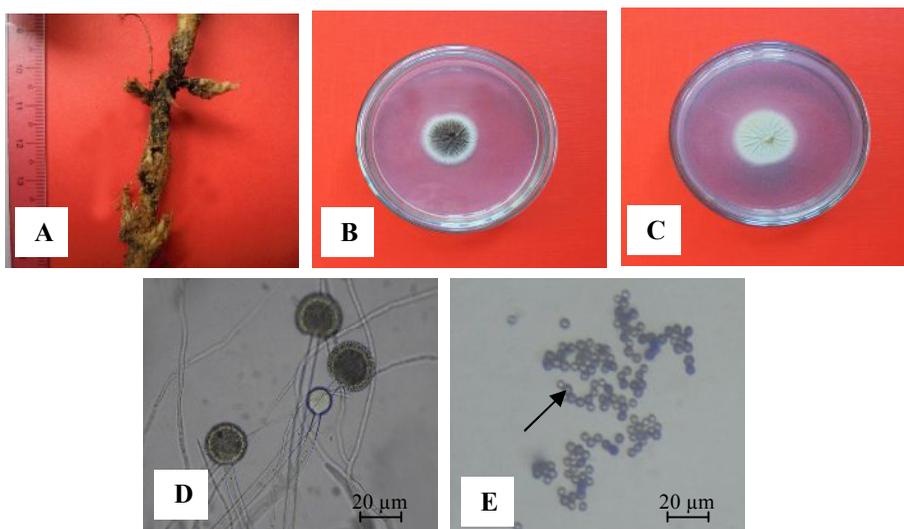


Figure 6. A. Leaf spot disease symptom infected on stem of groundnut (arrow)
 B. Surface colony characters of *Aspergillus* sp. (KYZN 05) strain on PDA medium (3 days)
 C. Reverse colony characters of *Aspergillus* sp. (KYZN 04)
 D. Hyphae with pycnidia of *Aspergillus* sp.
 E. Conidia of *Aspergillus* sp. (arrow)

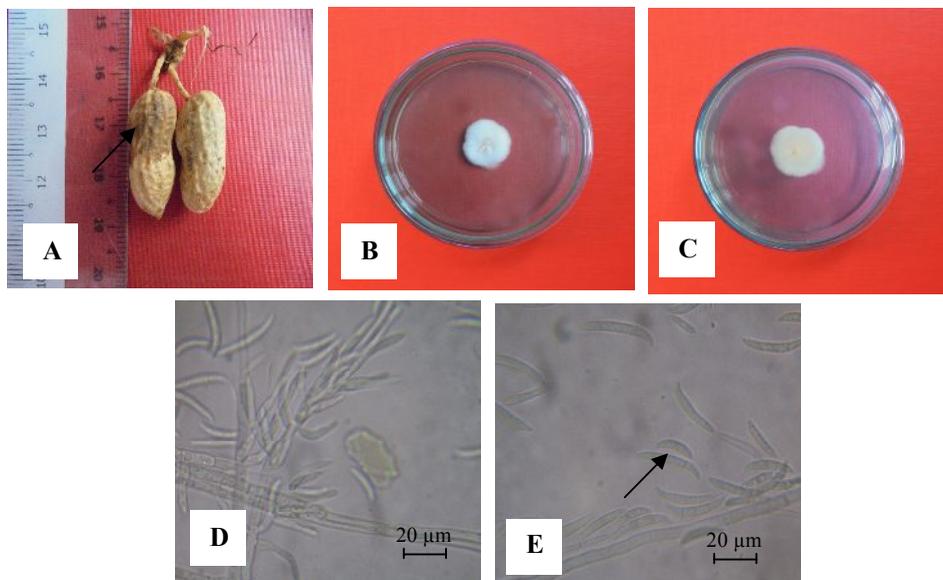


Figure 7. A. Leaf spot disease symptom infected on pod of groundnut (arrow)
 B. Surface colony characters of *Fusarium* sp. (KYZN 06) strain on PDA medium (3 days)
 C. Reverse colony characters of *Aspergillus* sp. (KYZN 06)
 D. Hyphae with pycnidia of *Aspergillus* sp.
 E. Conidia of *Aspergillus* sp. (arrow)

Table (2) Characteristics of pathogenic fungi isolated from infected parts of *Arachis hypogaea* L.

Isolated strain	Pathogenic fungi	Macroscopical characters	Microscopical characters	Diseases
KYZN 04	<i>Macrophomina</i> sp.	White to brown or gray and darken at mature. Reverse remains dark.	Pycnidia are dark to grayish, globose or flattened globose. Conidiophores are septate. Conidia are hyaline, elliptical or oval.	Root rot (root)
KYZN 05	<i>Aspergillus</i> sp.	Pale yellow colony at firstly and turn to black. Reverse remains pale yellow.	Hyphae are septate. Conidiophores are upright, simple. Conidia are spherical	Crown rot (stem)
KYZN 06	<i>Fusarium</i> sp.	White and cottony. Reverse remains pale	Hyphae are septate. Conidiophores branch. Conidia with transverse	Pod rot (pod)

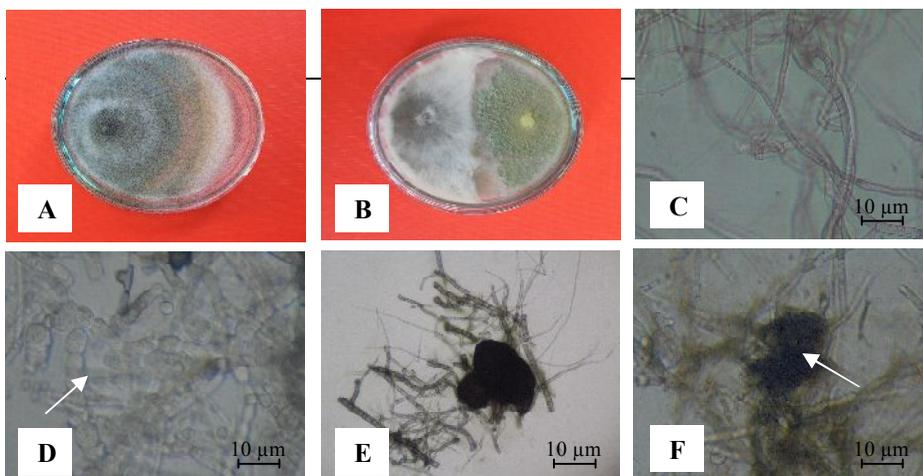


Figure 8. Antagonistic activity of KYZN 01 on *Macrophomina* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Macrophomina* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Macrophomina* sp. (arrow)
 E. Regular growth of pycnidia of *Macrophomina* sp. in control culture
 F. Degrading pycnidia of *Macrophomina* sp. (arrow)

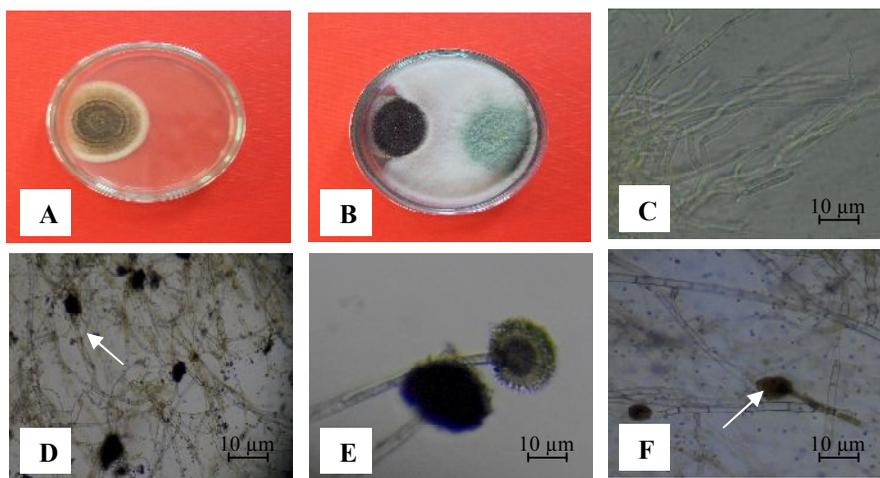


Figure 9. Antagonistic activity of KYZN 01 on *Aspergillus* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Aspergillus* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Aspergillus* sp. (arrow)
 E. Regular growth of conidial head of *Aspergillus* sp. in control culture
 F. Conidial head showed malformation of *Aspergillus* sp. (arrow)

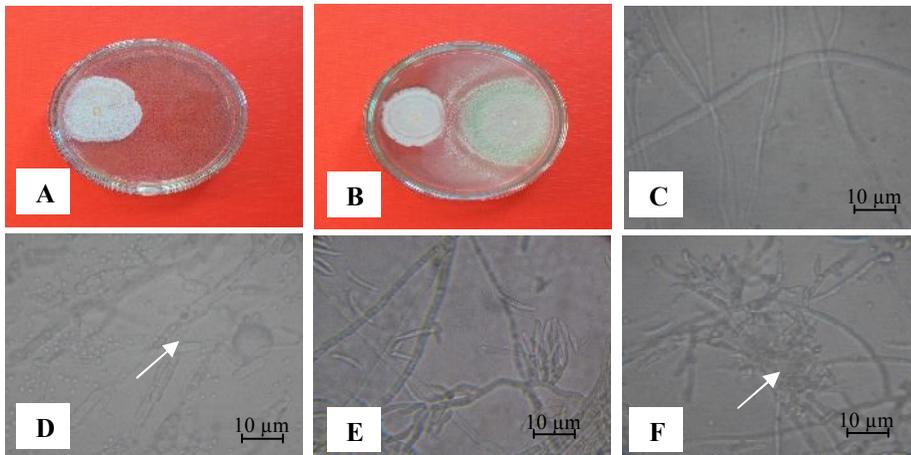


Figure 10. Antagonistic activity of KYZN 01 on *Fusarium* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Fusarium* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Fusarium* sp. (arrow)
 E. Regular growth of conidial head of *Fusarium* sp. in control culture
 F. Conidial head showed malformation of *Fusarium* sp. (arrow)

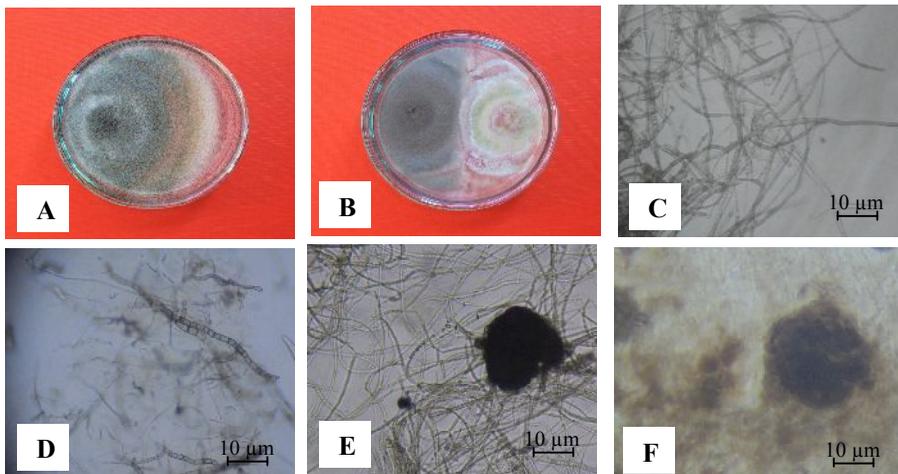


Figure 11. Antagonistic activity of KYZN 02 on *Aspergillus* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Macrophomina* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Macrophomina* sp. (arrow)
 E. Regular growth of conidial head of *Macrophomina* sp. in control culture
 F. Conidial head showed malformation of *Macrophomina* sp. (arrow)

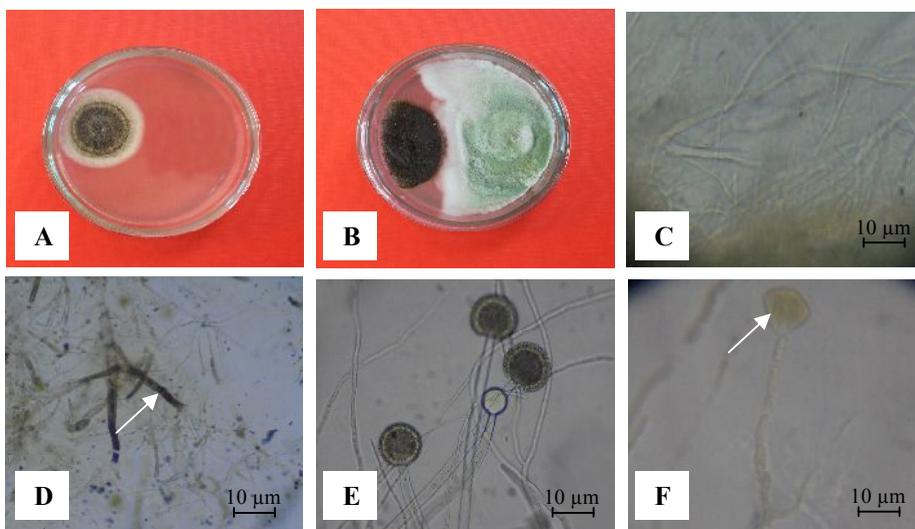


Figure 12. Antagonistic activity of KYZN 02 on *Aspergillus* sp. after dual cultures for 6 days on PDA medium

- Control of fungus mycelium
- Antagonistic interactions between *Trichoderma* strain and *Aspergillus* sp.
- Healthy mycelium with regular normal growth in control culture
- Malformation of fungal hyphae of *Aspergillus* sp. (arrow)
- Regular growth of conidial head of *Aspergillus* sp. in control culture
- Conidial head showed malformation of *Aspergillus* sp. (arrow)

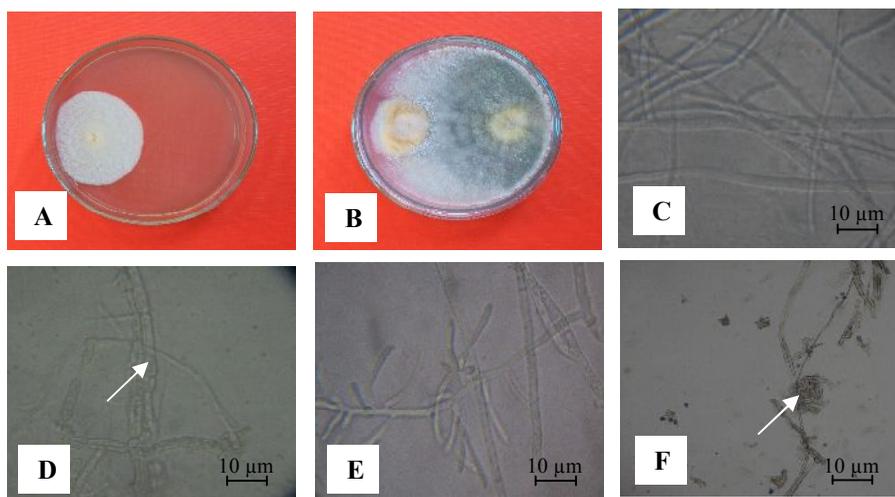


Figure 13. Antagonistic activity of KYZN 01 on *Fusarium* sp. after dual cultures for 6 days on PDA medium

- Control of fungus mycelium
- Antagonistic interactions between *Trichoderma* strain and *Fusarium* sp.
- Healthy mycelium with regular normal growth in control culture
- Malformation of fungal hyphae of *Fusarium* sp. (arrow)
- Regular growth of conidial head of *Fusarium* sp. in control culture
- Conidial head showed malformation of *Fusarium* sp. (arrow)

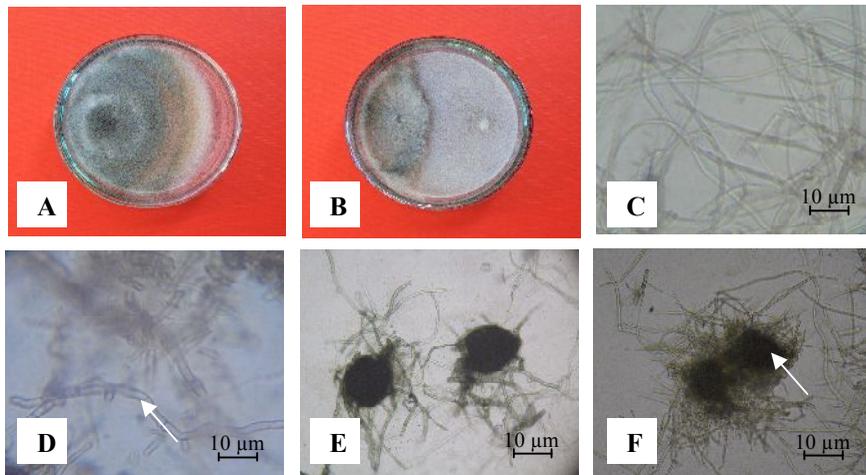


Figure 14. Antagonistic activity of KYZN 03 on *Macrophomina* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Macrophomina* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Macrophomina* sp. (arrow)
 E. Regular growth of conidial head of *Macrophomina* sp. in control culture
 F. Conidial head showed malformation of *Macrophomina* sp. (arrow)

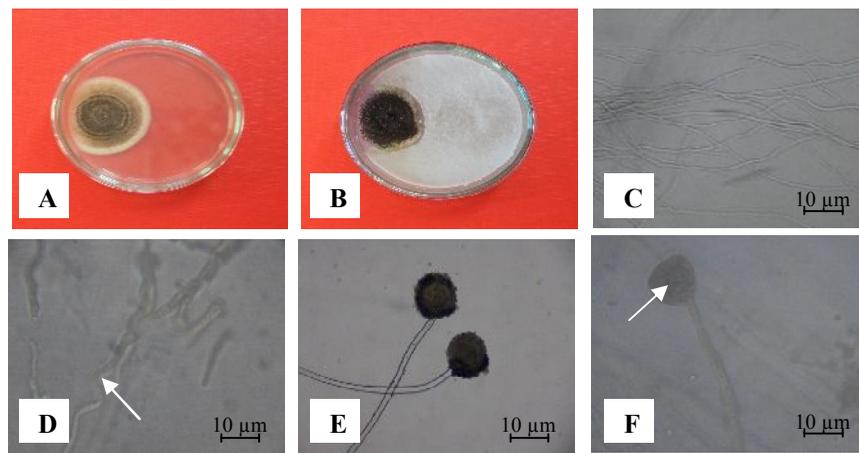


Figure 15. Antagonistic activity of KYZN 03 on *Aspergillus* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Aspergillus* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Aspergillus* sp. (arrow)
 E. Regular growth of conidial head of *Aspergillus* sp. in control culture
 F. Conidial head showed malformation of *Aspergillus* sp. (arrow)

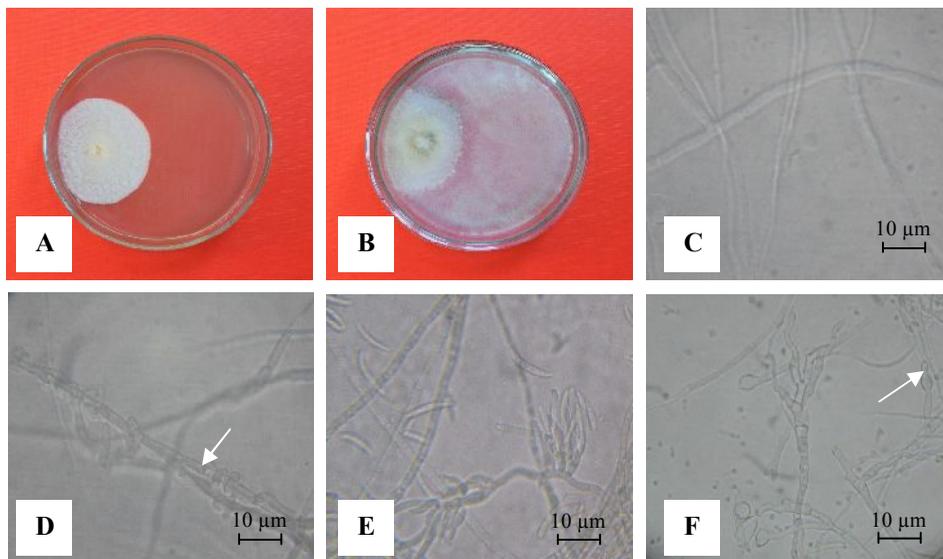


Figure 16. Antagonistic activity of KYZN 03 on *Fusarium* sp. after dual cultures for 6 days on PDA medium
 A. Control of fungus mycelium
 B. Antagonistic interactions between *Trichoderma* strain and *Fusarium* sp.
 C. Healthy mycelium with regular normal growth in control culture
 D. Malformation of fungal hyphae of *Fusarium* sp. (arrow)
 E. Regular growth of conidial head of *Fusarium* sp. in control culture
 F. Conidial head showed malformation of *Fusarium* sp. (arrow)

Table (3) Antagonistic activity of KYZN 01 against mycelia growth of pathogenic fungi

Pathogenic Fungi	Control	Test	Percent Inhibition
<i>Macrophomina</i> sp.	6.5	2	69%
<i>Aspergillus</i> sp.	3	1.5	50%
<i>Fusarium</i> sp.	3	1	66%

Table (4) Antagonistic activity of KYZN 02 against mycelia growth of pathogenic fungi

Pathogenic Fungi	Control	Test	Percent Inhibition
<i>Macrophomina</i> sp.	6.5	2.5	61%
<i>Aspergillus</i> sp.	3	1.5	50%
<i>Fusarium</i> sp.	2.5	1.3	48%

Table (5) Antagonistic activity of KYZN 03 against mycelia growth of pathogenic fungi

Pathogenic Fungi	Control	Test	Percent Inhibition
<i>Macrophomina</i> sp.	6.5	1.8	72%
<i>Aspergillus</i> sp.	3	1.3	56%
<i>Fusarium</i> sp.	3	1.2	60%

Table (6) Percent inhibition by *Trichoderma* strain isoaltes after 6 days of inoculation in dual cultures

<i>Trichoderma</i> strains	Test Pathogens			Mean
	<i>Macrophomina</i> sp.	<i>Aspergillus</i> sp.	<i>Fusarium</i> sp.	
	KYZN 04	KYZN 05	KYZN 06	
	PIRG (%)			
KYZN 01	69	50	66	61.7
KYZN 02	61	50	48	53
KYZN 03	72	56	60	62.7

Discussion and Conclusion

In this research, the antagonistic activities of *Trichoderma* were studied on pathogenic fungi of *Arachis hypogaea* L. The fungus antagonists, three strains of *Trichoderma* were isolated from the rhizospheric soil and infected leaves of *Arachis hypogaea* L.. *Macrophomina* sp., *Aspergillus* sp. and *Fusarium* sp. were isolated from infected parts of root rot, crown rot and pod rot.

In the present investigation, Rose Bengal Medium was used for *Trichoderma* strains. Johnson & Curl (1972) stated that Rose Bengal Medium is a suitable selective medium for *Trichoderma* strain. KYZN 01 grew rapidly in that medium and the colonies were white green, bright green to dull green and microscopical characters were septate mycelium, upright conidiophore, single phalides and obovoid conidia. These characters of KYZN 01 is accordance with description provided by Barnett (1955). PDA medium was used for KYZN 02 and KYZN 03 according to the methods of Johnson & Curl (1972). The macroscopical characters of KYZN 02 and KYZN 03 was white to grayish colony with yellowish reverse

colonies. The microscopical characters were branched conidiophores, phialide in false whorls with usually more than 2-3 and possessing globose conidia. The characters were agreement with the statement of Bhale *et al.* (2013) and Barnett (1955). Therefore, the KYZN 01, KYZN 02 and KYZN 03 were identified as *Trichoderma* strain.

In the present research, pathogenic fungi KYZN 04 was isolated from root rot symptom. In the macroscopical character, the mycelia of KYZN 04 was upright and white to brown or gray and darken at mature. In the microscopical characters, pycnidia are dark to grayish, becoming black at mature; globose or flattened globose. The pycnidia bear septate conidiophores. Conidia are single celled, hyaline and elliptic or oval. The above data are in agreement with those of Kaur *et al.* (2012). Amusa *et al.* (2007) also stated that *Macrophomina* sp. can cause root rot, wilt, leaf blight and stem blight in leguminous plants. Therefore, the fungus KYZN 04 was *Macrophomina* sp..

The results have shown that the macroscopical and microscopical characters of KYZN 05 strain was septate and hyaline mycelia. Vesicle subglobose, phialide sterigmata, biseriate conidia arose from the tip of phialide and spherical, 3-4 μm in diameter. Therefore, the fungus of KYZN 05 was *Aspergillus* sp. which supported by Bessay (1952).

The KYZN 06 fungus was white and cottony on PDA medium at room temperature (25°C) and pH 6.5-7.0 after 3-7 days. The microscopical characters of KYZN 06 was septate hyphae. Conidiophore was long or short, simple or branched bearing terminal phialide from which conidia bears on the upper surface. Bessay (1952) described that *Fusarium* sp. is characterized by hyphae septate, hyaline. Three types of spores produce namely macroconidia, microconidia and chlamyospore. The macroconidia are curved, may be found 3-5 septate, mostly 3 septate. Microconidia are borne on simple phialides arising laterally and abundant, oval-ellipsoid, straight to curved non septate. Chlamyospores are terminal or intercalary, arising singly or in short chains. Therefore, KYZN 06 was confirmed as *Fusarium* sp. according to report of Bessay (1952).

The level of antagonistic effects showed inhibition of fungal pathogens with varying effectiveness. It was based on the values of percent inhibition of radial growth (Thanh *et al.* 2014). The three strains of *Trichoderma* showed inhibition of fungal pathogen *Macrophomina* sp., *Aspergillus* sp. and *Fusarium* sp. with varying effectiveness. The direct confrontation of antagonistics against the pathogenic fungi. The inhibition

on pathogenic fungi due to antagonistic activities ranged from 50 to 69%, 48 to 61% and 60 to 72% by KYZN 01, KYZN 02 and KYZN 03, respectively. The maximum inhibitory activity of *Trichoderma* strain KYZN 01 was 69% against *Macrophomina* sp. and followed by 66% on *Fusarium* and 50% on *Aspergillus* sp.. *Trichoderma* strain KYZN 02 showed maximum inhibition against 61% on *Macrophomia* sp. and followed by 50% on *Aspergillus* sp. and 48% on *Fusarium* sp.. *Trichoderma* strain KYZN 03 had the highest effects against 72% *Macrophomina* sp. but minimum inhibition is in 60% on *Fusarium* sp. and 56% on *Aspergillus* sp.. The maximum inhibitory activity of all three strain of *Trichoderma* inhibited against *Macrophomina* whereas it inhibited against *Aspergillus* sp. at minimum scale. The maximum inhibitory activity of *Trichoderma* strain KYZN 03 was 62.7% (mean) against *Macrophomia* sp., *Aspergillus* and *Fusarium* sp.. KYZN 03 showed parasitic behavior against *Macrophomina* sp. by coiling around the host hyphae and degrading it.

Sreedevi *et al.* (2011) described that the antagonistic activities of *Trichoderma harzianum* and *Trichoderma viride* against on *Macrophomina phaseolina*. *Trichoderma harzianum* inhibited the growth of *M. phaseolina* upto 64.7% followed by *T. viride* 47%. In this investigation, KYZN 03 inhibited the growth of *Macrophomina* sp. and the highest percentage inhibition was (72%). *Trichoderma* sp. over grew the host resulting into complete degradation of the *Macrophomina* sp.. KYZN 01 and KYZN 02 gave minimum inhibition of *Macrophomina* sp. with rating 69% and 61%.

Sneha & Satya Prasad (2014) reported that the *Trichoderma* species inhibited the growth of oilseed-borne fungi like *Aspergillus flavus*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium oxysporium*, *Rhizopus nigricans*, *Penicillium chrysogenum* and *P. notatum*. In the present research, *Aspergillus* sp. was found to be abnormal growth of hyphae and conidial head under microscope due to attack of *Trichoderma* strains. KYZN 03 showed a good inhibition of *Aspergillus* sp. and gave the maximum percentage (56%). KYZN 01 and KYZN 02 showed the same inhibition condition (50%) on *Aspergillus* sp.

Khang *et al.* (2013) stated that the isolate *Trichoderma harzianum* were screened against *Fusarium* sp. following dual plate culture technique. The isolates of *Trichoderma harzianum* are found to be most effective and show the highest inhibition 71.69% and then the lowest inhibition is 50.91% in radial growth. In this study, KYZN 01, KYZN 02 and KYZN 03 isolates grew considerably faster on PDA in the same condition of culture. These

Trichoderma sp. isolates were able to inhibit the mycelial growth of *Fusarium* sp. by the range of 48% to 66%. Maximum inhibition zone (66%) was exhibited by the isolate KYZN 01 while the minimum (48%) inhibition was found by the isolate KYZN 02. Therefore, all of these *Trichoderma* strains have significantly inhibited on pathogenic fungi which infected on groundnut plants. Under the microscope, it was observed that hyphae of KYZN 02 and KYZN 03 coiled around hyphae of *Fusarium* sp. and finally segmentation of its mycelial tips.

In conclusion, three strains of *Trichoderma* were undertaken for the biological control of fungal disease in groundnut plants. Three strains of *Trichoderma* are potential to inhibit the growth of plant pathogens. The *Trichoderma* strains significantly inhibited the mycelium growth of plant pathogenic fungi and reduced disease severity in groundnut plants. Hence, it is recommended that *Trichoderma* strain can be used as effective biocontrol agents to control plant diseases.

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