BIBLIOGRAPHY

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ABSTRACT

The study was conducted at the Department of Plant Pathology at Benguet State University, La Trinidad Benguet from November 2011 to March 2012. The study aimed to Isolate and evaluate indigenous biological control agents against coffee rust for organic coffee production, and to identify and characterized effective biological Control agents against rust disease of Arabica coffee.

Result of the study showed that fungi and bacteria were isolated in different media. Isolates from different media was used in Bioassay in order to evaluate and characterize the effective Biological Control Agent against coffee rust on organic coffee production.

Eight fungi and eleven bacteria were isolated from symptomatic coffee plants that were obtained from coffee plant parts from twigs, leaves and flowers. Only 2 fungi were identified except for *Verticilliums.p.* and *Trichoderma*that was taken from Dr. Luciana Villanueva and Dr. Asuncion Nagpala. Furthermore, study was continuously develop in identifying bacterial isolates except for *Bacillus subtilis* (81.93, 73 and158) that was taken from Dr. Luciana Villanueva.The fungi identified were (05-F) Fusarium sp. and (06-F) *Penicillium sp.*

TABLE OF CONTENTS

Page

Bibliography……..…..……. i

Abstract……..……...…….. i

Table of Contents……. ii

INTRODUCTION…… 1

REVIEW OF LITERATURE…….. 3

MATERIALS AND METHODS…….… 7

RESULTS AND DISCUSSION……... 12

Isolation and Characterization
Of Biological Control Agents ……..... 12

a. Fungal Biological Control
Agent Isolates……...…… 12

b. Bacterial Biological Control
Agent Isolates…… 15

Bioassay on Detached Leaf …… 18

Leaf Disc Assay…….. 20

a. Number of Lesions on Leaf
Disc (After 15 days)……... 20

b. Diameter (mm) of Lesions on Leaf
Disc (After 12 days)…… 20

c. Number of Lesions on Leaf
Disc (After 15 days)……. 21

d. Diameter (mm) of Lesions on
Leaf Disc (After 12 Days…… 22

SUMMARY, CONCLUSION AND RECOMMENDATION……. 23

Summary……. 23

Conclusion…….. 23

Recommendation…… 23

LITERATURE CITED……. 24

APPENDICES……. 26

INTRODUCTION

Coffee is one of the most traded agricultural commodities in the world. It is accepted as the most important brewed beverages due to the stimulating effect on the human by its caffeine content. It ranks second to water (Pendergrast, 2009).

Coffee belongs to the genus Coffea L. of the family Rubiaceae. Coffee Arabica, and coffee Canephora known as "Robusta" are now cultivated throughout the coffee growing countries (Anonymous, 2003).Worldwide, Arabica coffee is the most important variety. It accounts for 72% of coffee production. Arabica coffee is early bearer, that after two years of transplanting it produce cherries. When generally managed and fully growned, one hectare farm could yield 1,000 kilograms of green beans.

The production of coffee in the Philippines according to the National Integrated RDE Agenda Programs (NIRDEAP) reached an all time high of 61,140 metric tons in the year 1992 and an all time low of 37,000metric tons in 1998. In fact, such performance of coffee in our industry was brought by the dry spells caused by El Niño phenomenon.

Coffee leaf rust caused by *Hemelieavastatrix*Berk& Br. on Arabica coffee is one of the important and classical diseases; it is a major disease that causes economic loss that has been reported from over fifty coffee growing countries (Bhat*et al*., 2000).

Biological control agents over coffee leaf rust is viewed as a progressive, environmentallyand more eco-logically friendly way of control. Use of pesticides to organismscausing diseases may pose many problems like toxic substances that might harm human and may essentially providepermanent environmental damage due to irreversible harmful effect to untargeted organisms and to ecological process. However, before releasing a biological control agent, it is important to determine its potential for

the control of rust and conservation of non- target organisms. Hence, the need for the evaluation of indigenous biological control agents against coffee rust will help the farmers to maintain the quality of their crops. It will also minimize economic losses, moreover, contributing to the restoration of biodiversity, and clean air.

The study aimed to:

1. Isolate and evaluate indigenous biological control agents against coffee rust for organic coffee production.

2. To characterize andidentify effective biological controlagents against rust disease of Arabica coffee.

The study was conducted at the Department of Plant Pathology Laboratory at Benguet State University from November 2011 to March 2012.

REVIEW OF LITERATURE

Climate Requirement of Coffee

Mc Mahon, et al (2002), cited that coffee Arabica requires temperature between 15°C to 24°C; trees withstand temperatures near 0°C (32°F) for only a short period. Continuously exposure temperature below freezing, trees suffer cold damaged and with considerable difficulty in recovering. However, temperature above 19.5°C (80°F) will tend to reduce flowering and fruiting, and temperature below 13°C (55°F) will cause cessation of growth and tree stunting.

Taxonomy of the Coffee Rust

Coffee leaf rust (*Hemilieavastatrix*) is one of the most feared pathogens to coffee growers. It is classified under class Pucciniomycetes, order Pucciniales and family Pucciniaceae (Conrad, 2009). According to Sangatanan (1986), *Hemilieavastatrix* is the causal agent that mostly produces only uredinia stage and is prevalent. However, telial and basidial are also observed sometimes.

Distribution and Magnitude of Damage Of Coffee Rust

Coffee leaf rust caused by the fungus *Hemelieavastatrix*is an obligate parasite, which occurs worldwide in coffee growing region (Bettencourt and Rodriguez Jr. 1988). It is a major disease of Arabica coffee causing economic losses that was reported from over fifty countries including India. In 1869, the fungus appeared in Ceylon now (Sri Lanka) were it infects the foliage and the young branches, which the fungus was exists in different physiological forms (Bhat*etal.,* 2000). The importance is largely responsible in

increasing again when the disease invaded the Latin America countries during 1970-1985 (Bhat, 2005).

Coffee is subjected to various problems that include the important diseases which result to reduced photosynthetic capacity of infected leaves and premature defoliation associated with high infection levels; also it reduced the heavy carbohydrate sink created by fruit limits which serves the amount of growth of woody tissue that gives rise to next crop season. Thereby, the following season's crop is reduced. In fact, it is estimated that the losses due to coffee leaf rust can seriously reach 30 to 80% annually, although 15 % is more typical (Kushalappa and Eskes,1989).

According to Mitchell (1988), coffee leaf rust was first to destroyed their crops in Brazil in 1970. The disease and its symptoms were first to observed in Sri Lanka and Ethiopia, the disease had become globally widespread by wind and rain and spores, lesions on the underside of plant.

Mechanism of Infection and Symptoms

Brown *et al* (1995) reported that the urediniospores will penetrate coffee through stomata and develop powdery orange pustules on the abaxial surface of leaves, resulting in impaired photosynthesis, premature defoliation, and reduced floral initiation constitute most of the damage. Arneson (2000), confirmed that Hemilieavastatrix survives primarily as dikaryotic(having pairs of haploid nuclei that divide in tandem), nutrient absorbing mycelium in the living tissues of the host. Hyphae are club-shaped with tips bearing numerous pedicels on with clusters of urediniospores are produced.

The coffee leaf rust was commonly small in shape, yellowish in spots. The disease appears on the lower surface of leaves that can produce powdery yellow to orange

spores. Although, die back was been characterize by drying of branches and twigs of the coffee plant. In severe cases, it can cause the leaves to fall off (Stoll, 2008).

Agrios (1988), cited that the symptoms of the disease are small circular spots, about 5mm in diameter, which are greenish yellow in the upper surface of the leaf and yellowish to orange on the leaf's lower surface. The spots may eventually become dry in the center of the leaf, turn to brownish and may even leaf galls off prematurely.

Management

Effective management of this major disease is important for sustained production and productivity of coffee. However, adopting cultural practices, planting resistance varieties and the application of contact and systemic fungicides are recommended for control measures (Anonymous, 1998). But in some instances, continuous use of fungicides may pose on many problems like toxicity to non target organisms that may involve the development of resistance in pathogen and ground water pollution (Daivasikamani and Govindarajan, 1989). In recent years they turn to shift in controlling the plant disease from regular use of pesticides to an alternate and more eco friendly bio pesticides and plant based production where, many fungi and bacteria has strains to act as a biological control agents. The indigenous strains of Bacillus subtilis and Pseudomonas flourescens appear to function as better antagonists in disease control as they will adapt to local conditions (Hanumanth*a et al.,* 1989).

Rangeshwaran and Prasad (2000) reported that P. flourescens is not much effective to work on biological agents of coffee leaf rust pathogen. Thus, present investigation was carried out to assess the antagonistic effect of *B.* subtilis and P.

flourescens. Although, bacterialantagonists were tested in vitro in vivo for their suppressing or controlling effect to the coffee leaf rust pathogen.

Since the fungicides were never used, the Hyperparasitic fungus Verticilliumhemilieaoccurs quite frequently and able to reduce the rust inoculums under high humid conditions. Under favorable conditions, thehyper parasite completely covers rust uredospores and lesion with white mycelia mass. Consequently, uredosores can be killed through necrosis of the rust lesions. In some instances, these Hyperparasites are organisms that parasitize other parasitic and are sometimes used as biological agents (Bhat, 2005).

MATERIALS AND METHODS

Collection and Isolation of Biological Control Agents

Arabica plantations in Benguetwere surveyed and randomly sample healthy anddisease Arabica coffee plants. Sampling was done in month of November to March, a period where heavy rust infection and leaf fall occur. Every epiphyte found associated to the coffee tissue specimens and not known as pathogen werepresumed asbeneficial or biological control agent. These were isolated with nutrient agar (NA) or potato dextrose agar (PDA). Pure cultures of each isolated biologicalcontrol agents were maintained forbioassay activities and characterization.

Cultural and Morphological Characterization

The isolated biological control agents were characterized in terms of the colony growth and development in culture media. The isolates were sampled, mounted in slides andexamined under the microscope to characterize the appearances of their microscope structures.

Laboratory Bioassay

Two techniques of leaf bioassay were done, the detached leaf and leaf disc assay.Separate trials were done for fungal biological controlagents from the bacterial biological control agent isolates.

Detached Leaf Assay. Healthy leaves of Arabica coffee were collected, washed with sterile distilled water, blot dried and placed in transparent polyethylene bags. The leaves were inoculated with dilutions of coffee rust uredospores, followed by spray of

biological control agents suspension, enclosed by tying the openings and incubated for 48 hours. Observations of symptom development in terms of number and size of lesions were done.

Bioassay trials detached leaves in the laboratory were laid in the completely randomized design (CRD) with three replications.

The treatments for the detached leaf assay were:

T_1 - Kocide

T_2 - Control (Plain H20)

T_3 - 01-F

T_4 - 01-B

T5 - *Verticillium sp.*

T_6 - *Trichoderma sp.*

Leaf Disc Assay.The leaves were washed with sterile distilled water and then cut into pieces toobtain leaf discs at size of 2.5x2.5cm. The leaf discs wereplaced in sterile Petri dishthat wereoverlaid with wet tissue paper, inoculated with dilutions of coffee rust uredospores, and subsequently sprayed with suspensions of biological control agents. The treated leaf discs were incubated for48 hours. Developments of uredospores from 48 hours of incubation were monitored up to when the leaves were still green, and when uredospores were visible.

Bioassaytrials on leaf discs werelaid in the completely randomized design (CRD) with three replications.

Biological control agentisolates as treatment were:

Bacterial Biological ControlAgent Isolates	Fungal Biological ControlAgent Isolates
T_1-01-B	T_1-Kocide (control)
T_2- 02-B	T_2-01-F
T_3-03-B	T_3-02-F
T_4-04-B	T_4-04-F
T_5-05-B	T_5-05-F (*Fusarium sp.)*
T_6-06-B	T_6-06-F*(Penicillium sp.)*
T_7-07-B	T_7-*Verticillium sp.*
T_9-31 (*Bacillussp.*)	T_8-*Trichoderma sp.*
T_{10}-73(*Bacillus sp.*)	T_9-*Trichoderma sp.*
T_{11}-94 *Flavobacterium sp.*	T_{10}-Kocide
T_{12}-158 *Pseudomonas sp.*	

Figure 1. Experimental set- up on Detached Leaf Assay

Figure2. Experimental set- up for Leaf Disc Assay

Data Gathered were:

1. Isolate as biological control agents. Collection of diseased and healthy coffee tissues was diagnosis to explore the presence of biological control agents against coffee rust.

2. Number of germinated and ungerminated spores of coffee rust (leaf discand detached leaf bioassay).These were recorded starting from 48 hours after inoculation. Lesions diameter were measured in millimeters.

3. Number and diameter of lesions on leaf disc and detached leaf.Inhibition of the biological control agents on the developments of the uredospores were assessed 15 days after inoculation.

4. Morphological and Cultural Characteristics of the Isolates. The cultural characterization was done by observing the colony growth and development on the culture media, while the morphological characterization was done through microscopic observations of the structures of the isolates.

RESULTS AND DISCUSSION

Isolation and Characterization ofBiological controlAgents

Fungal Biological Control AgentIsolates. There were six fungal biological control agents isolated and characterized. Most isolates in terms of cultural characteristics,grown in PDA had a light orange to orange pigment while other isolates produced yellow bluish green, light brown and white pigment in culture (Isolates 1 to 6). For morphological characteristics observed under microscope, most isolates revealed on oblate conidia (Table 1).

Table 1.Cultural and Morphological of Fungal BiologicalControl Agents.

	ISOLATES					
CRITERIA	01-F	02-F	03-F	04-F	Fusarium sp. (05-F)	Penicillium sp. (06-F)
Growth	Fast growing	Slow growing	Slow growing	Slow growing	Slow growing	Fast growing
Colony color	Orange	Light yellow	From white to Light orange	White	Orange	Green
Presence of septa on mycelim	Present	Present	Present	Present	Present	Present
Shape of conidia	Round & oblate	oblate	oblate	Rectangular	Macro-sickle Micro-oblate	Round
Color of conidia	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
Size of conidia	1.75-5.5 µm	1.75-3.0 µm	2.0-5.25 µm	1.75-3.75 µm		

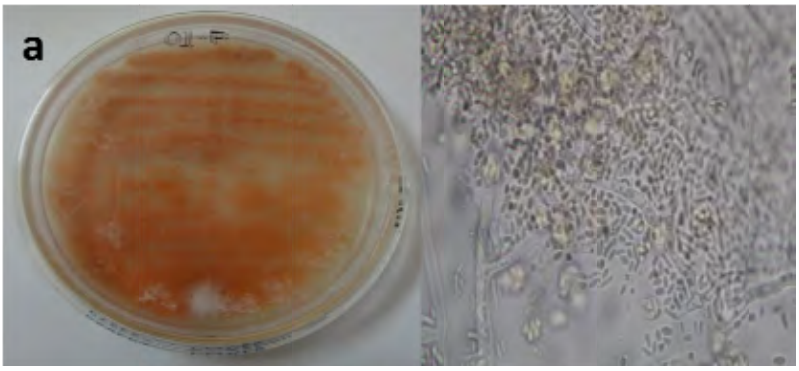

Figure 3. Isolate 01-F Colony (Left) Structures (Right)

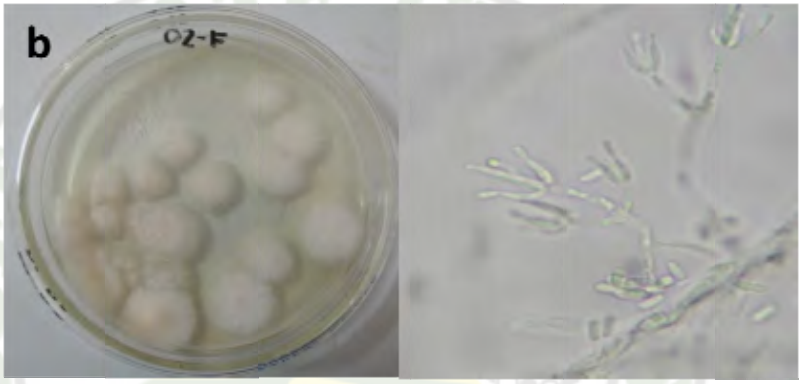

Figure 4. Isolate 02-F Colony (Left) Structures (Right)

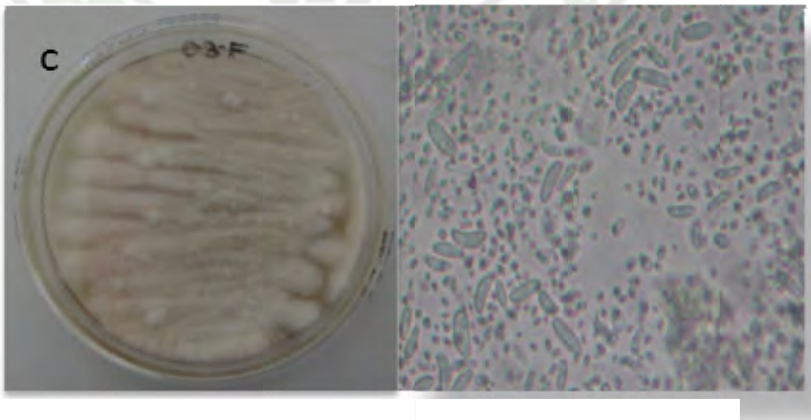

Figure 5. Isolate 03-F Colony (Left) Structures (Right)

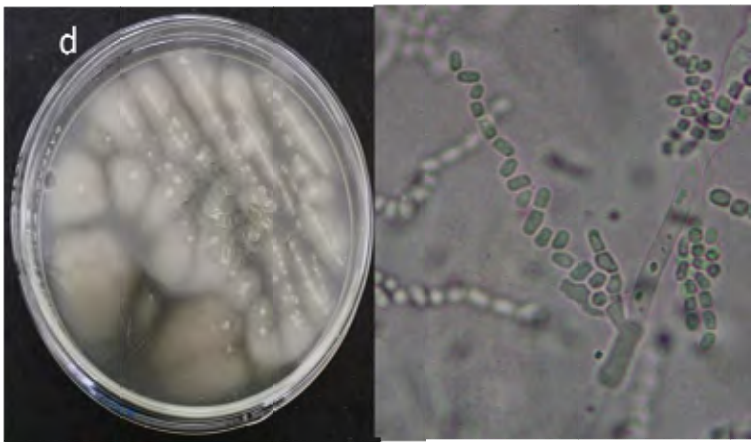

Figure 6.Isolate 04-F Colony (Left) Structures (Right)

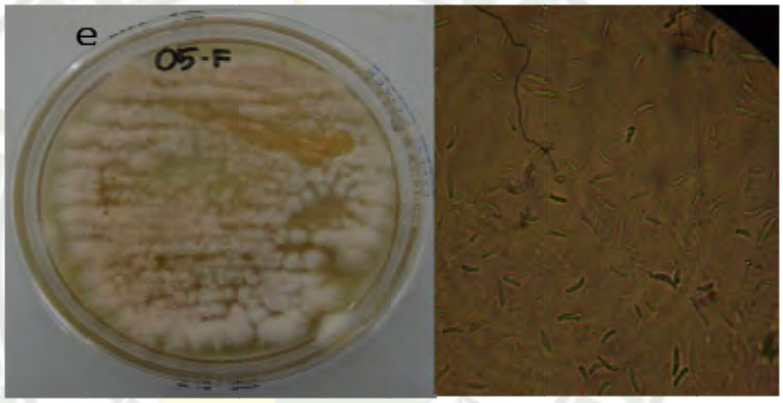

Figure 7.*Fusarium sp*. colonyIsolate

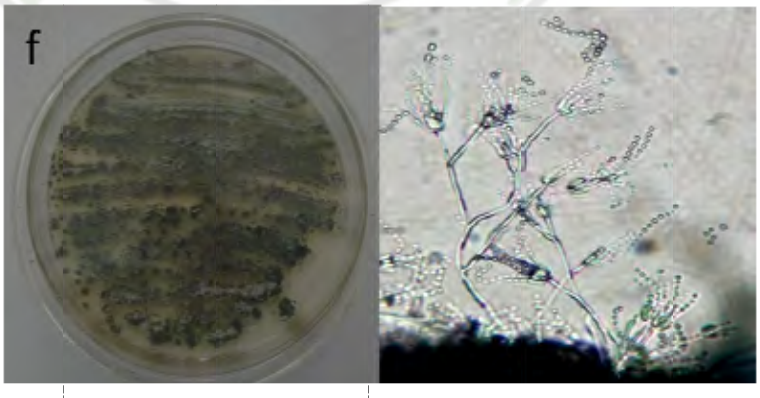

Figure 8.*Penicillium sp*. colonyIsolate

Bacterial Biological Control Agent Isolates. Thebacterialbiological controlagents were isolated in nutrient broth (NA). Pure cultures obtained were subjected to cultural and morphological characterization. Cultural characteristics of bacterial isolates that was grown in NA had a flat, irregular, and white in terms of their form, elevation, and color, while other isolated produced with wavy, lobate and entire margins (Isolates 1 to 7). For morphological characteristics, microscopic examination revealed to rod shaped, cylindrical and cocci shaped like (Table 2).

Table 2. Characteristics of Bacterial Biological ControlAgent Isolates

ISOLATES	FORM	ELEVATION	MARGIN	COLOR	SHAPE	GRAM (+\-)
01-B	Irregular	Flat	wavy (undulate)	White (shiny)	rod	-
02-B	Irregular	Flat	wavy (undulate)	White (shiny)	rod	+
03-B	Irregular	Flat	lobate	transparent	rod	+
04-B	Circular	Convex	wavy (undulate)	Yellow (shiny)	rod	-
05-B	Circular	Convex	Entire	white	cocci	-
06-B	Circular	Umbonate	lobate	white	rod	-
07-B	Circular	convex	Entire	white	cylindrical	-

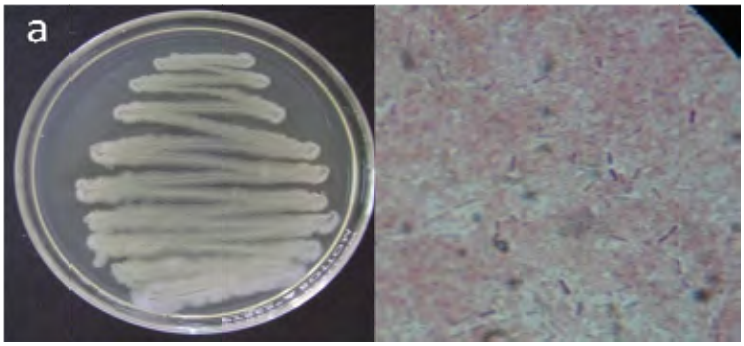

Figure 9. Isolate 01-B Colony (Left) Structures (Right)

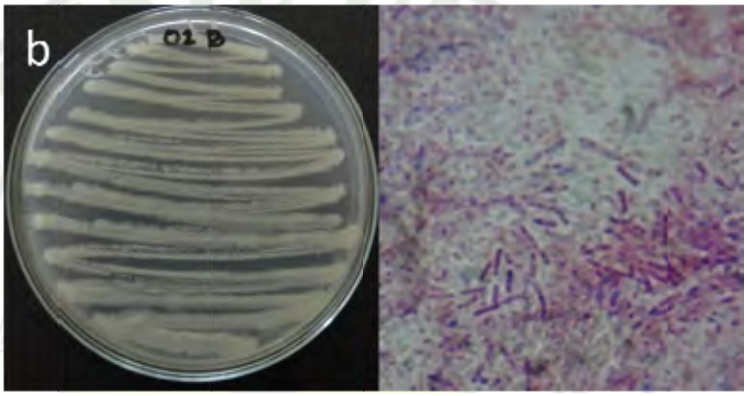

Figure 10. Isolate 02-B Colony (Left) Structures

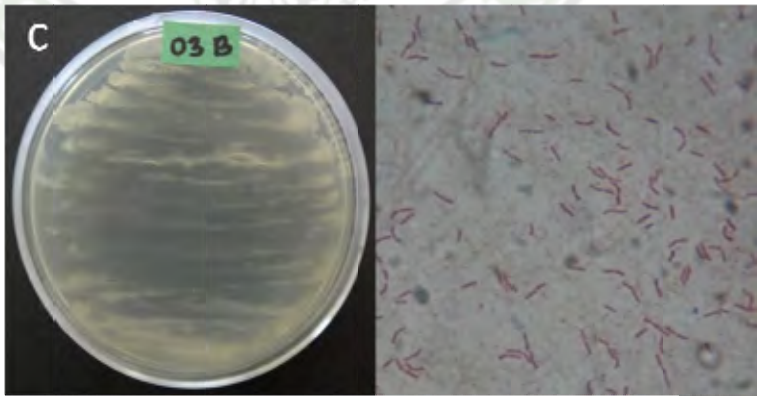

Figure 11. Isolate 03-B Colony (Left) Structures

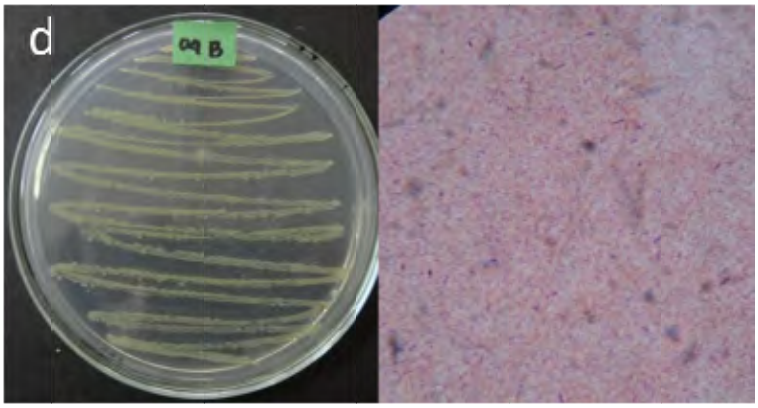

Figure 12. Isolate 04-B Colony (Left) Structures

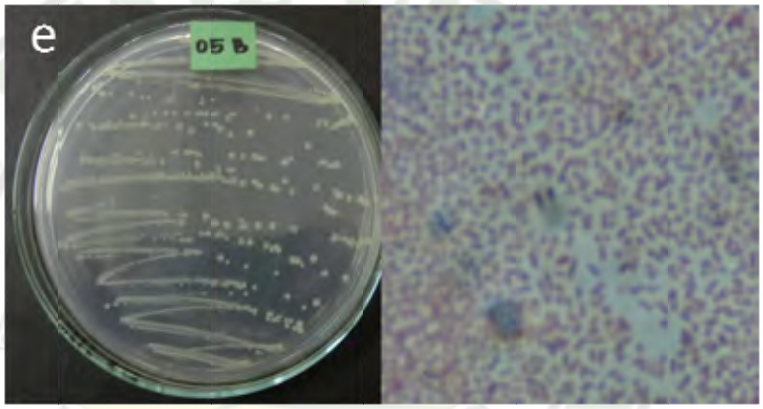

Figure 13. Isolate 05-B Colony (Left) Structures

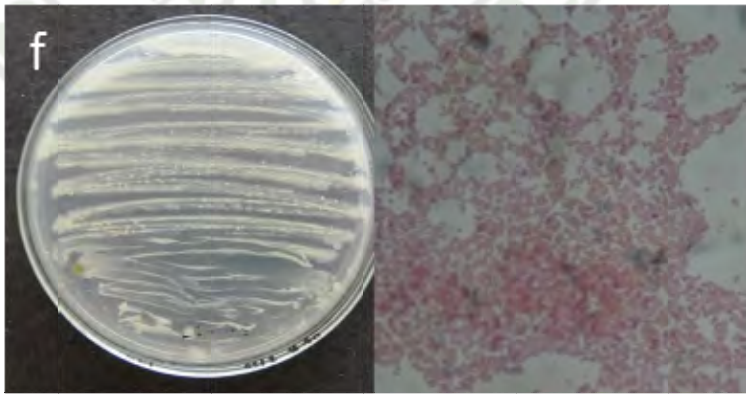

Figure 14. Isolate 06-B Colony (Left) Structures

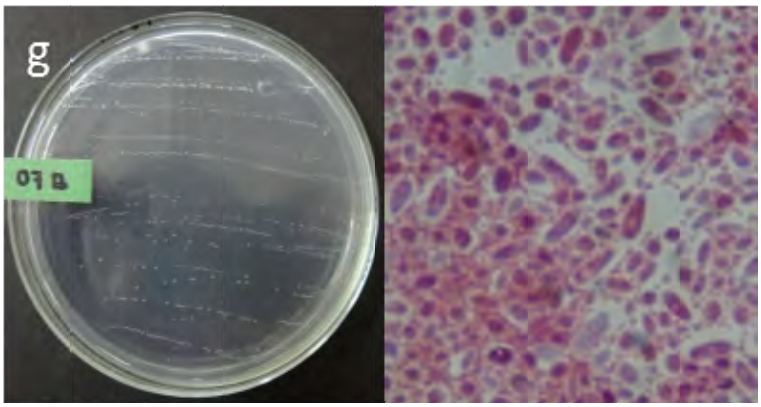

Figure 15. Isolate 07-B Colony (Left) Structures

Bioassay on Detached Leaf

Percentage spore germination inhibition in Table 3 shows the reaction of coffee rust to the biological agent.The control which is plain water had the highest percentage of spore germination inhibition with a mean of 99.6,while the lowest was from those treated with kocidewith a spore germination inhibition of 87.1.No significant difference was recorded in the percent inhibition of sporegermination on detached leaf.

Length of germ tube (µm) Table 3 shows that the length of germ tube of germinated spores. The highest germ tube length of 3.7µm was obtained from those treated with kocide and 01-F, and the lowest mean of 2.2 µm was obtained from those treated with 01-B. Statistically there were no significant difference among the treatments

Table 3.Reaction of Coffee Rust of Biological Control Agent Against on Detached Leaf.

TREATMENTS	SPORE GERMINATION INHIBITION (%)	LENGTTH OF GERM TUBE (µm)	
		Actual	Transformed
Kocide 87.1	3.67	3.7	
Control (Plain H20)	99.6	3.23	3.2
01-F	98.3	3.72	3.7
01-B	78.4	2.22	2.2
Verticillium sp.	98.3	2.41	2.4
Trichoderma sp.	98.2	2.52	2.5

Number of lesions on Detached Leaf (After 18 days) eighteen days after inoculation, the number of lesions on detached leaf was counted. Most of the isolates, plain water and kocide treatments had a lesion of which is different from those treated with 01-F with a mean of 2. (Table 4) reveals that there were no significant differences in the number of lesions after eighteen days of inoculation.

Diameter of lesions on detached leaf (After 18 days).The diameter of lesions on detached leaf revealed that the highest was 2.1mm that was obtained from 01-F, and the lowest with a mean of 1.2 mm were *Verticillium sp*. There were no significant differences among the treatments (Table 4).

Table 4.Reaction of Coffee Rust of Biological Control Agent Against on Detached Leaf.

TREATMENTS	NO. OF LESIONS		DIAMETER OF LESIONS (mm)	
	Actual	Transformed	Actual	Transformed
Kocide0.33	1.00	1.33	1.2	
Control (Plain H20)	1.00	1.00	3.33	1.7
01-F	4.67	2.00	5.00	2.1
01-B	1.00	1.00	2.33	2.0
*Verticillium sp.*0.66		1.00	1.33	1.2
*Trichoderma sp.*1.33		1.00	2.67	1.5

Leaf Disc Bioassay

Number of lesions on Leaf disc (After 15 days)

Table 5 shows the number of lesion on leaf disc assay after 15 days of inoculation. Treated leaf disc with 04-, 06-B, 07-B, 73, 94 and 158 had the same means of 2.0, and mean of 1.50 was obtained from those treated with, 01-B, 02-B, 03-B, 05-B and 31 isolates including the control. There was no significant difference.

Diameter (mm) of Lesions on Leaf disc (After 12 days)

The diameter of lesions on leaf disc is shown on table 5. The highest was obtained from those treated with 03-B isolates with a mean of 2.79mm and the lowest was from those treated with 01-B isolate with a mean of 1.41. Statistical analysis revealed no significant differences.

Table 5. Effect of Bacterial BiologicalControlAgentsAgainst Coffee Rust on Leaf Disc Assay

TREATMENTS	NO. OF LESIONS	DIAMETER OF LESIONS (mm)
Control (Plain water)	1.5	2.04
01-B (Bacteria)	1.5	1.41
02-B (Bacteria)	1.5	2.33
03-B (Bacteria)	1.5	2.79
04-B (Bacteria)	2.0	2.21
05- B (Bacteria)	1.5	2.29
06-B (Bacteria)	2.0	2.04
07-B (Bacteria)	2.0	2.0
31(*Bacillussp.*)	1.5	2.58
73(*Bacillus sp*.)	2.0	1.71
94 *Flavobacterium sp.*	2.0	1.79
158 Pseudomonas *sp.*	2.0	2.04

Number of Lesions on Leaf disc (After 15 days)

Table 6 shows that the highest number of lesion on leaf disc was obtained from isolate 02-F with a mean of 2.11 followed by *Verticillium sp.* with a mean of 1.83 and the lowest were from *Trichoderma sp*. with a mean of 1.20. However, statistical analysis revealed no significant differences on the number of lesions on leaf disc after 15 days of inoculating.

Diameter of Lesion on Leaf disc (After12 days)

Twelve days of inoculation, the diameter of lesions on leaf disc was counted. The 04-F has the highest diameter of lesion on leaf disc with a mean of 2.67 and kocide has the least mean diameter of lesion on leaf disc with a 1.46. In addition, there was a highly significant difference in the mean of the diameter of the lesion leaf in terms of statistical analysis (Table 6).

Table 6. Effect of Final Biological Control Agent Against Coffee Rust on Leaf disc

TREATMENTS	NO. OF LESIONS	DIAMETER OF LESIONS (mm)
Control	1.33	1.58[c]
01-F	1.55	1.71[b]
02-F	2.11	2.33[a]
03-F	1.37	2.42[a]
04-F	1.47	2.67[a]
05-F (*Fusarium sp.)*	1.63	2.6[a]
06-F(Penicillium sp.)	1.43	2.1[a]
Verticillium sp.	1.83	2.12[a]
Trichoderma sp.	1.20	2.37[a]
Kocide	1.37	1.46[c]

Means with the same are not significantly different at 5 DMRT

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The study was conducted at the Department of Plant Pathology Laboratory at Benguet State University, La Trinidad, Benguet from November 2011 to March 2012. The study aimed to isolate and evaluate indigenous biological control agent against coffee rust for organic Arabica coffee production, and to characterize and identify and effective biological control agent against rust disease of Arabica coffee.

There were 8 fungi and eleven bacteria isolated and presumed as Biologicalcontrol Agents Against the coffee rust. Two of the fungal isolates were identified as *Fusarium sp.* and *Penicilliumsp.*Two of the bacterial isolates were identified as Bacillus strains, a *Flavobacterium*and *Pseudomonas sp.* isolate.

Conclusion

Result of the bioassays on leaf discs and detached leaves were generally not significantly different. The data on the effectively of the fungal and bacterial isolates as biologicalcontrol agents against coffee rust not conclusive.

Recommendation

Based on the findings of the study, the following are recommended:

1. Evaluation of the isolated biologicalcontrol agent against coffee rust shall be done on seedlings.

LITERATURE CITED

AGRIOS, G . N. 1988. Plant Pathology 3rd ed. University of Florida, Gainesville. Pp.466 – 488.

ANONYMOUS. 2003. Coffee guide. Central coffee.Research institute, Chickmagalur, Karnataka, India.P. 200.

ANONYMOUS. 1998. A compendium on pests and diseases of coffee and their managemen in India. Coffee board research department, central coffee research institute,Chickmagalur,Karnataka, India. P.67.

ARNESON, P. A. 2000. Coffee rust.The plant health instructor. DOI:10.1094/PHI-1-2000-0718-02.Retrieved August 13, 2011 fromhttp://www:apsnetorg/edcenter/in/coffee rust.aspx.

BHAT, S. S. 2005. Integrated management of coffee leaf rust. Retrieved August 13,2011: fromhttp://india coffee. Org/new letter/2005/apr/cover-story-2.Html.

BHAT, S. S., NAIDU. R.S., DAIVASIKAMANI. and NIRMALA KANNAN. 2000. Integrated disease management in coffee. In: IPM system in agriculture. Cash crops.Vol./ 6.

BETTENCOURT, A. J. and C. J. RODRIGUEZ. 1988. Principles and practice of coffee breeding forresistance to rust and other diseases. In: CLARKE R.J. , MACRAER, eds, coffee: London : EL Sevier applied science. Pp. 199-234.

BROWN, J., WHAN, J., KENNY, M. and P. MERRIMAN. 1995. The effect of coffee leaf rust onfoliation and yield of coffee in Papua New Guinea. Crop protection.14(7);589-592.

CONRAD, J. 2009. Rust fungi. The Backyard Nature RetrievedAugust 15, 2011 from http://www. Backyard nature.Net/f/rusts.html.

DAIVASIKAMANI, S. and T. S. GOVINDARAJAN. 1989. Field efficacy of a new systemic fungicide PP 523 5% SC (Hexaconazole) research. 19: 65-70.

HANUMANTHA, B. T., GOVINDARAJAN, T. S. and NIRMALA KANNAN. 1989. Comparative efficacy of four fungicides for control of coffee rust. Journal of Coffee Research. 19:57-64Retrieved August 15, 2011 fromhttp://en. Wikipedia.Org/wiki/coffee

KUSHALAPPA, A. C., A. B. ESKES. 1989. Advances in coffee rust research. Ann. Rev. Phytopathol.27:503-31.

MITCHELL, H. W. 1988. Cultivation and Harvesting of the Arabica Coffee Tree: Agronomy. Ed. R. J CLARKE. New York:ElSeveir Applied Science.

MC MAHON, M. J., KOFRANEK, A. M., RUBATZKY, V. E. 2002. Hartmann's plant science.Growth, developments and utilization of cultivated plants 3rd ed. Upper, Saddle River, New Jersey. Pp325.

PENDERGRAST, M. 2009.Tea and Coffee Trade Journal. Retrieved December 29, 2009 from http://en.wikipedia.org/wiki/Coffee

RANGESHWARAN, R. and R. D. PRASAD, 2000. Isolation and evaluation of rhizospheric bacteria for biological control of chickpea wilt pathogens. Journal of biological control, 14:9-15.

SANGATANAN, P. D. 1986. Agricultural arts 4.Home economics and livelihood technology services. School graduate studies, West Visayas State College, Ilo-ilocity. P.64.

STOLL, G. 2008. Workshop on demand workshop on module.Integrating OISAT into agricultural training and ext. services.

APPENDICES

APPENDIX TABLE 1.Actual Percentage (%) Inhibition of Spores Germination On Detached Leaf (After 48 hrs)

TREATMENTS	REPLICATION			TOTAL	MEAN
	I	II	III		
T$_{o}$Kocide	96.39	81.47	83.54	261.4	87.13
Tı Control	99.46	99.68	99.55	298.69	99.56
$T_2$01- F	98.87	98.91	97.15	294.93	98.31
$T_3$02- F	97.54	39.19	98.4	235.14	78.38
T_4 *Verticillium sp.*	98.15	98.39	98.25	294.79	98.26
T_5Trichoderma sp.	98.41	96.77	99.44	294.62	98.21
TOTAL	196.56	514.41	576.33	1679.57	559.86

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	5	1121.32	224.26	1.10^{ns}	3.11	5.06
Error	12	2440.13	203.34			
TOTAL	17	3561.45				

*^{ns}= not significant at 5% level CV = 15.28%

APENDIX TABLE 2.Actual Length of Germ tubes of Germinated Spores on Detached Leaf (µm) after 48 hours.

TREATMENT S	REPLICATIONS				
	I	II	III	T	M
T_0Kocide	6	29	9	44	14.66
T_1 Control	9	9	12	30	10.00
T_2 01-F	3	36	10	49	16.33
T_3 01-B	2	5	7	14	4.67
T_4Verticillium sp.	10	4	3	17	5.67
T_5Trichoderma sp.	9	5	4	18	6.00
TOTAL	39	88	45	172	57.33

APENDIX TABLE 2.1Transformed Length of Germ tubes of Germinated Spores on Detached Leaf (µm) after 48 hours.

TREATMENT S	REPLICATION				
	I	II	III	T	M
T_0Kocide	2.55	5.43	3.08	11.06	3.67[a]
T_1 Control	3.08	3.08	3.54	9.7	3.23[a]
T_2 01-F	1.87	6.04	3.24	11.15	3.72[a]
T_3 01-B	1.58	2.35	2.74	6.67	2.22[a]
T_4Verticillium sp.	3.24	2.12	1.87	7.23	2.41[a]
T_5Trichoderma sp.	3.08	2.35	2.12	7.55	2.52[a]
TOTAL	15.4	21.37	16.39	53.36	17.79

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	5	371.78	74.36	0.91[ns]	3.11	5.06
Error	12	978.66	81.56			
TOTAL	17	1350.44				

*[ns]= not significant at 5% level CV = 94.46%

APPENDIX TABLE 3.Actual Number of Rust Lesion on Detached Leaf Assay(After 18 days)

TREATMENT S	REPLICATIONS				
	I	II	III	T	M
T_0Kocide	0	0	1	1	0.33
T_1 Control	0	2	1	3	1.00
T_2 01-F	0	5	9	14	4.67
T_3 01-B	0	1	2	3	1.00
T_4Verticillium sp.	0	0	2	2	0.66
T_5Trichoderma sp.	4	0	0	4	1.33
TOTAL	4	8	15	27	9

APPENDIX TABLE 3.1TransformedNumber of Rust Lesion on Detached Leaf Assay(After 18 days)

TREATMENT S	REPLICATIONS				
	I	II	III	T	M
T_0Kocide	0.71	0.71	1.22	2.64	.88
T_1 Control	0.71	1.58	1.22	3.51	1.17
T_2 01-F	0.71	2.35	3.08	6.14	2.05
T_3 01-B	0.71	1.22	1.58	3.51	1.17
T_4Verticillium sp.	0.71	0.71	1.58	3.00	1.00
T_5Trichoderma sp.	2.12	0.71	0.71	3.54	1.18
TOTAL	5.67	7.28	9.39	22.34	7.44

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	5	37.83	7.57	1.55^{ns}	3.11	5.06
Error	12	58.67	4.89			
TOTAL	17	96.50				

*^{ns}= not significant at 5% level CV = 39.25%

APPENDIX TABLE 4.Actual Diameter (mm) of Rust Lesions on Detached Leaf Assay (After 18 days)

TREATMENTS	REPLICATIONS				
	I	II	III	T	M
T_0Kocide	0	0	4	4	1.33
T_1 Control	0	8	2	10	3.33
T_2 01-F	0	7	8	15	5.00
T_3 01-B	0	2	5	7	2.33
T_4Verticillium sp.	0	0	4	4	1.33
T_5Trichoderma sp.	8	0	0	8	2.67
TOTAL	8	17	23	48	16

APPENDIX TABLE 4.1TransformedDiameter (mm) of Rust Lesions on Detached Leaf Assay (After 18 days)

TREATMENTS	REPLICATIONS				
	I	II	III	T	M
T_0Kocide	0.71	0.71	2.12	3.54	1.18
T_1 Control	0.71	2.92	1.58	5.21	1.74
T_2 01-F	0.71	2.74	2.92	6.37	2.12
T_3 01-B	0.71	1.58	2.35	4.64	1.55
T_4Verticillium sp.	0.71	0.71	2.12	3.54	1.18
T_5Trichoderma sp.	2.92	0.71	0.71	4.34	1.45
TOTAL	6.47	9.37	11.8	27.64	9.21

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	5	28.67	5.73	0.46^{ns}	3.11	5.06
Error	12	149.33	12.44			
TOTAL	17	178.00				

*^{ns}= not significant at 5% level CV = 132.10 %

APPENDIX TABLE 5.ActualNumber of Lesions on Leaf Disc using Bacterial Isolates(After 15 days).

TREATMENTS	REPLICATION			
	I	II	T	M
T_1 Control	2	1	3	1.5
T_2 01-B	1	2	3	1.5
T_3 02-B	2	2	4	2
T_4 03-B	1	2	3	1.5
T_5 04-B	2	2	4	2
T_6 05-B	1	2	3	1.5
T_7 06-B	2	2	4	2
T_8 07-B	3	1	4	2
T_9 31	2	1	3	1.5
T_{10} 73	2	2	4	2
T_{11} 94	2	2	4	2
T_{12} 158	1	3	4	2
TOTAL	21	22	43	21.5

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	11	.91	0.08	0.17^{ns}	2.72	4.22
Error	12	5.76	0.48			
TOTAL	23	6.67				

*^{ns}= not significant at 5% level CV = 43.57 %

APPENDIX TABLE 6.Actual Diameter (mm) of Lesions on Leaf Disc using Bacterial Isolates(After 12 days).

TREATMENTS	REPLICATION			
	I	II	T	M
T_1 Control	1.75	2.33	4.08	2.04
T_2 01-B	1.67	1.16	2.83	1.41
T_3 02-B	3.08	1.58	4.66	2.33
T_4 03-B	2.58	3.0	5.58	2.79
T_5 04-B	2.83	1.58	4.41	2.21
T_6 05-B	2.58	2.0	4.58	2.29
T_7 06-B	2.33	1.75	4.08	2.04
T_8 07-B	2.58	1.41	3.99	2.0
T_9 31	2.75	2.41	5.16	2.28
T_{10} 73	1.58	1.83	3.41	1.71
T_{11} 94	1.58	2.0	3.58	1.79
T_{12} 158	2.08	2.0	4.08	2.04
TOTAL	27.39	23.05	50.44	25.22

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	11	3.10	0.28			
Error	12	3.49	0.29	0.97^{ns}	2.72	4.22
TOTAL	23	6.59				

*^{ns}= not significant at 5% level CV =25.64 %

APPENDIX TABLE 7.Actual Number of Lesions on Leaf Disc using Fungal Isolates (After 15 days)

TREATMENTS	REPLICATION			
	I	II	T	M
T_1 Control	1	1	3	1
T_2 01-F	2	1	3	2
T_3 02-F	2	3	4	2
T_4 03-F	1	1	3	1
T_5 04-F	1	2	3	1
T_6 05-F	1	2	3	2
T_7 06-F	1	2	3	1
T_8 *Verticillium sp.*	2	1	4	2
T_9 *Trichoderma sp.*	1	1	3	1
T_{10}Kocide	1	2	3	1
TOTAL	13	16	29.5	14.5

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	9	1.32	0.15			
Error	10	2.36	0.24	0.62^{ns}	3.02	4.95
TOTAL	19	3.68				

*^{ns}= not significant at 5% level CV = 32.02%

APPENDIX TABLE 8.Actual Diameter (mm) of Lesions on Leaf Disc using Fungal Isolates (After 12 days)

TREATMENTS	REPLICATION			
	I	II	T	M
T_1 Control	1.41	1.75	3.16	1.58
T_2 01-F	1.66	1.75	3.41	1.71
T_3 02-F	2.37	2.33	4.66	2.33
T_4 03-F	2.58	2.25	4.83	2.42
T_5 04-F	2.42	2.91	5.33	2.67
T_6 05-F	2.62	2.58	5.2	2.6
T_7 06-F	1.75	2.42	4.17	2.1
T_8 *Verticillium sp.*	2.33	1.91	4.24	2.12
T_9 *Trichoderma sp.*	2.16	2.58	4.74	2.37
T_{10}Kocide	1.33	1.58	2.91	1.46
TOTAL	20.63	22.06	42.69	21.35

ANOVA TABLE

SV	DF	SS	MS	FC	TABULATED F	
					5%	1%
Treatment	9	3.25	0.36			
Error	10	0.67	0.07	5.14**	3.02	4.95
TOTAL	19	3.92				

**= Highly significant at 1% level CV = 12.42%