TRICHODERMA-BASED MANAGEMENT OF CLUBROOT DISEASE OF CRUCIFERS

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ABSTRACT

The study was conducted at the Bureau of Plant Industry in Baguio and in the Municipalities of Buguias and Bakun in Benguet to evaluate the best substrate for the growth of *Trichoderma koningii* strain, identify the best application method of *Trichoderma koningii* for the control of clubroot disease of crucifers and compare the efficacy with Pentachloronitrobenzene against clubroot disease of crucifers. The results reveal that: 1) *Trichoderma koningii* strain, cultured in rice hull produced the maximum spores with average count of 6.8×10^7 in 30 days at temperatures ranging from 21 to 22^{0}C stored under ambient condition and 10 days at temperatures 26 to 30^{0}C under plastichouse; 2) the application of *Trichoderma koningii* strain as biological control cultured in rice hull substrate significantly suppressed clubroot disease severity of cabbage by 72.20%. This was applied as basal three weeks before planting with rate of 1tbs/planting hole. *Trichoderma koningii* strain at half rate of pure culture grown in potato dextrose agar diluted in 16 li water applied as soil drenched one day before planting had 57.92%. In terms of yield, the application of *Trichoderma koningii* applied as basal produced heavier weights of cabbage of 25.33 tons/ha but was significantly comparable when applied as drenched with weights of 19.03 tons/hectare; 3) On- farm trials conducted in a clubroot severely infected farms in two barangays of Buguias, Benguet namely; Loo (cabbage) and Bad-ayan (Chinese cabbage) applied with *Trichoderma koningii* strain cultured in rice hull substrate significantly suppressed the clubroot disease severity by 54 to 56 %. The same treatment raised soil pH from acidic to moderately acidic and produced heavier weights of cabbage and Chinese cabbage heads.

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RATIONALE

Vegetables are important in the diet of every Filipino because it is the source of vitamins minerals and other nutrient elements. According to report, the vegetables industry in the Philippines has the highest contribution to the country's economy with the highest income per unit area and time (DA-BAR, 2004). With this scenario plant pests need to be suppressed to maintain the quality and abundance of food.

Among the most important constraints in vegetable production is the damage and considerable yield losses caused by plant diseases including soil-borne pathogens and plant parasitic nematode. More importantly, the case of soilborne pathogens is their capacity to reduce significant yield and quality of vegetable crops. The pathogens are particularly challenging because they can survive in soil for many years ((Koike et al., 2003). Among the economically important soilborne pathogens include *Psuedomonas, Plasmodiophora, Fusarium and Rhizoctonia.*

Clubroot caused by the pathogen *Plasmodiophora brassica* is a serious disease of brassica crops in most production areas around the world having caused severe losses to vegetable crops such as cabbage, cauliflower, broccoli and Chinese cabbage. While considered strictly a disease of brassica it is known to cause diseases on other families such as *Agrotis stolonifera* (creeping bent grass), *Fragaria spp.*and *Doctylis glomerata* (orchard grass).

In the Cordillera Region, specifically in Benguet and Mountain Province vegetable growers claimed that yield losses of about 75 to 85% on crucifer crops is caused by *Plasmodiophora brassicae* or clubroot disease and about 50% or more on potato caused by bacterial wilt .

Brassicae such as cabbage, Chinese cabbage, cauliflower, broccoli and lettuce are among the top major vegetable commodities grown in the Cordillera specifically in Benguet and Mountain Province including some areas in the Visayas and Mindanao.

As it stands today, farmers still rely on chemical fertilizers and pesticide to guarantee their farm produce. Such inputs to agriculture contributed significantly in the improvement of farm productivity and quality over the pass decades. However, these contribute to environmental pollution caused by extensive use and misuse of agrochemicals had lead to considerable change in people's attitude towards the use of pesticide in agriculture. Added to this is the cost of high rising

farm inputs such as agrochemicals and causing them to use alternative measures to cope up with burden. Hence, there are major concerns on strategies in managing the soil borne pathogens such as the use of beneficial microorganism *Trichoderma* as alternative approach.

The effectiveness of biological such as the Genus *Trichoderma* has been demonstrated not only to check soilborne pathogens but also degrades some group of pesticides that are highly persistent in environment (Harman **et al.**, 2004). In South America, they apply the fungus *Trichoderma* against witches-broom and frosty pod fungus, the main disease of cacao in Brazil and Peru, respectively (Mukerji et al., 1998). Successful works also showed that organic fertilizer rich in microorganisms such a *Trichoderma* is a good control of the disease "mal del alluelo" caused by *Rhizoctonia sp.* (Chet **et al.**, 1980). Reports also showed that potato cyst nematode using *Trichoderma* BSU Isolate suppressed the population to about 64-78% using potato variety Donald (Mangili et al., 2004).

Considering the benefits derived from the beneficial microorganism other strains of *T. koningii* could be integrated with other control approaches against important soilborne pathogens such as the clubroot disease of crucifers, Fusaruim wilt, plant parasitic nematodes of strawberry and bacterial wilt of potato, hence the study.

OBJECTIVES

1. To identify the best substrate/s for the growth of *Trichoderma koningii* strain
2. To verify the best application method for *T. koningii* strain against clubroot disease of crucifers.
3. To determine the efficacy of *T. koningii* strain cultured in rice hull against clubroot disease crucifers in farmers field.

REVIEW OF LITERATURE

Substrates in the spore production of the ***Trichoderma*** is important because it will affect the potency According Jenkins and Ebeling (1985), *Oryza sativa*, the hulls contained 4.5% lignin, 65.47% volatiles, 17.86 % ash, 16.67% fixed carbon, 40.96% C, 4.30% H, 35.86% O, 0.40% N, 0.02% S, 0.12% Cl, and undetermined residue. Further they claimed that fungi are responsible for the biodegradation of lignin in wood and other materials and they are remarkable in their ability to degrade a wide variety of environmental pollutants (Aust, 1995).

In 1984 it was reported that 38% of the 710 hectare planted to crucifers and inspected from 1989 to 1994 had clubroot disease (Mariano et al. as cited by Tad-awan, 2000). Further Tad-awan (2000) estimated that with an average production of cabbage from clubroot-free areas at 55 t/ha, a 20% yield loss to the 38% of the 710 ha or 269.8 ha of clubroot infected areas at a price of 5pesos/kg, almost 15 million pesos/cropping would have been due to clubroot disease.

Soilborne pathogens clubroot cause by *Plasmidiophora brassicae* can significantly reduce yield and quality of vegetable crops. These agents make up a diverse group. These fungi, which are multicellular microorganisms, cause most soilborne vegetable diseases and are considered the most important group. The fungal pathogens survive in the soil as saprobes on host debris or on other types of organic matter present in the soil (Koike et al., 2003).

Among the most important soil borne pathogens causing considerable losses in vegetable production worldwide include *Psuedomonas spp.*, *Plasmodiophora* spp., *Pythium, Fusarium* spp., *Verticilium dahli* (Lazarovits, 2001).

The clubroot disease occurs when the soilborne pathogen invades the plant root system. The infected roots become swollen, forming galls or clubs that interfere with the plant nutrient supply. In mild cases wilting and stunting occurs and severe cases, plant dies (Porth, 2005). The chief concern related to clubroot is the durability and life span of the resting spore. They may remain capable of initiating new infection for as long as twenty years. Thus once the field becomes infected it will remain infected for as long as twenty if no attention is made. The disease can be spread easily to clubroot disease-free areas through farm implements and could be transmitted by plant materials human, containers and vehicles.

Management strategies concerning the soilborne pathogen has been the interest of most scientists worldwide. Disease management of clubroot includes crop rotation, use of resistant variety, organic amendments, biological control, cultural and chemical.

The fungicide has been used for several years to treat infected fields with the clubroot. However, most require high application rather making them prohibitively

expensive. In addition, many fungicides have given inconsistent control and show adverse environmental effect due to the misuse and excessive uses (Howard, 2007). At the moment the Pentachloranitrbenzene is the most widely use but for the control of clubroot disease but only effective when integrated with other control strategies.

To date, biological control is accepted as one of the main concerns in disease management of vegetable crops because of the sustainability and the many benefits derived. Foremost is the environmental concerns, risk and sustainability (Aust, 1995). He further claimed that in the field of biological control the Genus *Trichoderma* is widely used in small commercial farms to suppress the population of soilborne pathogens.

The application of *Trichoderma* in the soil minimizes the use of chemicals because it does not function for control of pests but also enhanced the growth and development of plants which ultimately increase resistance and tolerance against pests. (http://wjow.soilfertility.com/trichoderma/English/index.shtml).

Fungal species of genus *Trichoderma* occur worldwide and can be isolated from soil, decaying woods and other forms of organic materials. They are generally mycoparasites which results in penetration of the cell wall of the host fungus and utilize the cellular contents, hydrolytic enzymes such as chitinase, glucanases and proteases, which partially induced before direct contact with the host (Hjeljord and Transmo, 1998).

The genus *Trichoderma* consists of five species and differs in the morphological features of the conidia and phialides. The microorganism has been studied as an organism for biodegradation, being able to degrade organochlorine pesticide, chlorophenols and other insecticides such as DDT, endosulphan and aldrin; herbicides such as Trifluralin (Aust, 1995). The *Trichoderma* mycoparasitism combine process such as nutrient competition (Chet, 1987) the secretion of anti fungal metabolites (Lorito et al., 1996) and formation of morphological changes such as coiling around the host and develop an appressorium like structure use in attacking the host (Lu et al., 2004).

Trichoderma is a kind of anaerobic, facultative fungus which can be found naturally in large numbers of agricultural soils and other media. They have the capacity to adapt to different environmental conditions thus the organism can be utilized in different soil, crops, climate and technological processes. Further it obtains its nutrients from fungi which it breaks down into organic matter. The

growth rate is fast making itself established in soil and control diseases. It does not appear pathogenic to any plants, however it can parasitize, control and kill many fungi, nematodes and phytopathogens that attack and destroys crops. It is an essential microorganism for soil and crops due to its unfathomable value to agriculture. It is also considered a soil conditioner since it makes the nutrients present in the soil available for plant growth and development (Cooks and Baker, 1996). *Trichoderma koningii* has various strains that are mutant that are more virulent that could work with a wide array of crop pests. The microorganism makes the plant resistant to pests that are present in the soil and it helps increase yield making nutrient available in the soil for growth and development of the crops.

The advantage of using biocontrol to combat plant diseases such as those present in the soil does not leave residues hazardous to the environment and to humans. The Application of *Trichoderma* in the soil minimizes the use of chemicals because it does not only function for control of pests but also enhanced the growth and development of plants which ultimately increase the resistance or tolerance against pests attack (http://wjow.soilfertility.com/trichoderma/English/index.shtml.)

Findings of Mangili et al. (2005), show that *Trichoderma* BSU isolate is effective against cyst nematode *Globodera rostochiensis* on potato with spore count of 10^7 using fine rice bran substrate at one tablespoon or approximately 10 grams per planting hole. The cyst nematode population reduced to about 64 – 78%. This was incorporated with chicken manure three weeks before planting the potato seed tubers.

STUDY 1. GROWTH OF *TRICHODERMA KONINGII STRAIN* ON DIFFERENT SUBSTRATES

OBJECTIVES

1. To evaluate different substrates for mass production of *Trichoderma*
2. Identify substrate/s that can produce the maximum spores in a short period.
3. Determine the economics of using the different substrates

RATIONALE

Trichoderma is among the biocontrol agents used by some of the highland vegetable growers to control soilborne diseases and even used as decomposer.

For the previous years the mass production of *Trichoderma* is being done by using fine rice bran. Based on experienced by Bureau of Plant Industry, Baguio National Crop Research an Development Center, the use of the substrate to produce maximum spores takes more than 35 to 60 days depending on the temperature under ambient condition. The temperature of 22 to 25^{0}C is favorable for the production of spores. Under Benguet condition the required temperature is hardly met during the cold and rainy months. It was therefore the aim of this study to test other substrates locally available.

METHODOLOGY

Six substrates representing 6 treatments were evaluated at the BPI-BNCRDC laboratory. These were placed in autoclavable transparent plastic bags at 300 grams each added to 200 ml distilled water and sealed with a rubber band before sterilized using the autoclave. The substrates were inoculated with a 10-day old pure culture of the *T. Koningii* strain cultured in a potato dextrose agar (PDA). The treatments were assigned using the complete randomize design (CRD) with 7 replication. The treatments were the following:

Treatments:

The substrates were stored under ambient condition for 30 days and spore count was monitored 30 days after inoculation.

The daily temperatures were recorded during the study period. Two sets of experiment were conducted from July to September 2007.

Data gathered:

1. **Number of spore count.** One tablespoon approximately 10 grams from each substrate was placed in bottle containing 100 ml distilled water after which the solution was stirred thoroughly. The solution was decanted from the bottle and filtered with the use of a mesh cloth. From the supernatants, one ml was obtained using a piston-driven air displacement pipette and dispensed in the hemocytometer to count for the spores under the microscope. This was monitored 30 days after spore inoculation in the substrates.
2. **Daily temperature.** This was recorded every day during the trail period.

RESULTS AND DISCUSSION

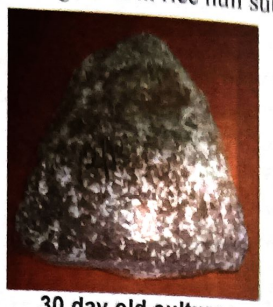
Table 1 presents the substrates used and the corresponding spore counts 30 days after spore inoculation. Results revealed that spore production of *T. koningii* strain using the different substrates have significant differences.

During the first and second experiments *T. koningii* strain cultured in rice hull produced the highest counts of spores followed by fine rice bran, and course rice bran with respective of 6.7 x 10^7, 6.1 x 10^6, 6.3 x 10^6 and 6.9 x 107, 6.1 x 10^6, 6.6 x 10^6. Lower spore counts were obtained from mushroom compost alone and mushroom compost + garden soil + chicken manure with 1:1:1 ratio with counts of $5.3x10^5$, $4.6x10^5$, $4.8x10^4$ and $5.3x10^5$, $4.6x10^5$, $5.0x10^5$, respectively.

The high spore production of the *T. koningii* strain *in rice hull could be* favorable as nutrient source than the other substrates. However, Jenkin and Ebelin (1985) reported that (*Oryza sativa*), the hulls of rice contains 4.5% lignin, 65.47% volatiles and other undetermined residue. They claimed that the *Trichoderma* are responsible in degrading the lignin in wood and use them as source of food nutrient which could be the reason for favoring the rice hull for the production of spores than the other substrates.

Fig. 1. *Trichoderma* pure culture and mycelial growth in rice hull substrate.

Pure culture



30 day old culture

The average temperature during the trial period ranged from 21 to 22^{0}C which is below the required temperature (25 to 30^{0}C) for the favorable growth of the microorganism (Begoudi et al, 2006). This may have affected the longevity in the production maximum spores. However when the same culture was stored under plastic house at temperatures ranging from 26-30^{0}C the maximum spores of 10^7 was produced in 10 days.

Table 1. Spore count of *Trichoderma koningii* strain per 10g substrates 30 days after spore inoculation.

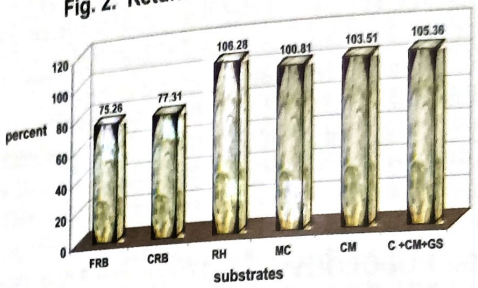
Treatments	Spore Count/10gm substrate		
	1st experiment	2nd experiment	Average
1. Rice hull	6.7×10^{7a}	6.9×10^{7a}	6.8×10^7
2. Fine rice bran	6.1×10^{6b}	6.1×10^{6cb}	6.1×10^6
3. Coarse rice bran	6.3×10^{6b}	6.4×10^{6b}	6.3×10^6
4. Mushroom compost	5.3×10^{5c}	5.3×10^{5c}	5.3×10^5
5. Chicken manure	4.5×10^{5e}	4.6×10^{5e}	4.5×10^4
6.compost + chicken manure + garden soil (1:1:1)	4.8×10^{4d}	5.0×10^{4c}	4.9×10^4
CV%	24.31	29.60	

Means with the same letters are not significantly different at 5% DMRT

In terms of return on cash expense (ROCE), higher profits were obtained form rice hull (106.28%), mushroom compost + chicken manure + garden soil (105.36%) and chicken manure with 103.51%. The least profit was from coarse rice bran and fine rice bran of 75.26 and 77.31%, respectively (Fig.1). The differences in ROCE are primarily due the cost of the substrates. Nevertheless, *Trichoderma koningii* strain favored the rice hull over the rests of the substrates.

Fig. 2. Return on cash expense of the different substrates.

Fig. 2. Return on cash expense of different substrates



STUDY 2. VERIFICATION TRIAL ON THE APPLICATION METHOD OF *TRICHODERMA KONINGII* STRAIN AGAINST CLUBROOT DISEASE OF CRUCIFERS.

RATIONALE

The use of biopesticide such as the biological agent for control of pests is gaining importance because of the tremendous price increase of farm inputs. Added to this, the consumers are becoming health conscious because of the many aspect effects of ailments brought about by the use pesticide (Cheng, 2002) This is further supported by the increasing demand for vegetables which are either raised as a result of integrated pests management (IPM), good agricultural practices (GAP) and organically grown ones.

When the beneficial microorganism *Trichoderma* was introduced to selected farmers in Benguet and Mountain Province with problems of potato cyst nematode and clubroot disease of crucifers it was suggested that other application methods such as spray be tested for ease of handling. Efficiency of the application methods being used has to be verified to help the users/farmers the ease of handling the *Trichoderma koningii* strain Hence, preliminary trials have been

conducted on- farm with farmer cooperators from the seed potato growers group with farms infected with clubroot disease prior to this experiment.

OBJECTIVES

1. To verify the best application method of *Trichoderma* against clubroot disease of crucifers in farmers field.
2. To determine the efficiency of the application methods in suppressing the clubroot disease
3. To determine their effects on yield

METHODOLOGY

The experiment was conducted at Bagtangan, Bakun, Benguet. Prior to the conduct of the experiment the area was planted to cabbage Scorpio, a susceptible variety to assess the extent of severity of the disease. During harvest the severity of clubroot disease incidence was assessed. Thirty pieces matured cabbage heads selected at random were uprooted washed and rated using the rating scale indices of 1-9 approved by the Fertilizer and Pesticide Authority (FPA). Based on the result, clubroot disease severity assessment rating ranged from 7 to 9 or severe cabbage root clubbing. This is done to make certain that the clubroot disease infection is present in the farm.

The area was thoroughly prepared into 160 plots each measuring 1 x 6 sq. m. The double row method of planting was used. The distance of planting was 30 x 30 between hills and rows. Before planting, chicken manure was applied in the planting holes with rate of 3 tons/hectare while urea was applied during hilling-up.

The cultural management practices such as spraying against insect pests, weeding, and irrigation were applied when necessary. Application of fungicide was applied against foliar diseases during the growing season.

There were three treatments each replicated five times. Eight plots comprised one replication. The random complete block design was used. The treatments are the following;

Treatments	Rate	Application Method
T_1 – Untreated (farmers practice)	-	-
T_2 – *T. koningii* strain (Pure	Half part of pure culture	Soil drenched

T_2 – *T. koningii* strain (Pure culture in PDA)	Half part of pure culture in petri plate/16 l water	Soil drenched
T_3 – *T. koningii* strain (in rice hull substrate)	1tbs/hole	Basal

The *Trichoderma koningii* strain cultured in rice hull was applied as basal three weeks before planting for both application methods. For pure culture in PDA a 10-day old culture of *T. koningii* strain was used to prepare the spore suspension. This was further mixed to a liter water solution and was poured in a 16 liter capacity sprayer provided with a mesh cloth. The solution was further diluted to 15 liter water and sprayer tank mixed before application. This was applied in the planting holes using a fine spray nozzle. One spray load approximately covered 250 planting holes. Chicken manure was applied at the rate of 3 tons/ha three weeks before planting, except for the untreated plots which was applied two weeks after transplanting the cabbage seedlings. Inorganic fertilizer triple-14 at 5 sacks per hectare was applied on *Trichoderma koningii* strain treated plots, while 11 sacks of T-14 per hectare were applied to the untreated plots. The reduction of inorganic fertilizer t-14 was based on preliminary studies conducted prior to this study.

DATA GATHERED

1. **Disease severity.** At harvest the whole plants were removed from the soil and roots were cutoff washed and scored for disease severity. Twenty samples were taken from each treatment replicate. The disease rating indices used is describe below:

Scale	Description
1	Normal roots
3	Minor lateral root clubbed ,0.5 cm diameter
5	Major lateral root clubbed , 1-2 cm diameter
7	Moderate root clubbed on tap root
9	Severe clubbed on roots and decaying/ed due to advance infection

2. **Disease control (%).** This was determined by using the scale below:

Disease rating in untreated

1. **Yield (tons/ha).** The weight and the number of cabbage heads were recorded.

RESULTS AND DISCUSSION

Table 1 shows the effect of the treatment methods based on the clubroot disease severity. Significantly, lower disease severity was obtained from the basal application of *Trichoderma* in rice substrate with score of 2.26 or minor lateral clubbed root, 0.5 cm diameter, compared when applied as drenched using pure cultured of *T. koningii* strain with 4.41 or almost more lateral roots are clubbed. The highest disease severity was from the untreated plots with score of 8.80 equivalent to severe clubbed roots and are decaying due to advance infection.

Table 1. Disease severity rating as affected by treatments.

Treatment	Mean
T_1 – Untreated	8.80[b]
T_2 - *Trichoderma* Pure culture (in PDA)	4.41[a]
T_3 - Trichoderma in rice hull substrate	2.62[a]
CV%	22.20

Means from the same letter do not differ significantly at 5% level DMRT

Fig. 3. Severity of clubroot on cabbage plants

Percentage control of clubroot disease severity on cabbage plants as affected by the treatments are presented in Table 2.

There were significant differences observed in the control of clubroot disease as affected by the greater percentage (72.20%) obtained from the application of *Trichoderma* grown in rice hull substrate applied as basal compared with the application as soil drenched with 57.92%. This could be due to the *Trichoderma* that was able to colonized in the roots of the cabbage and Chinese cabbage and inhibit the development of the causal organism *Plasmodophora brassicae*. This conforms to the observations of Hartman (2004) that only when *Trichoderma* is able to colonize the roots of diseased plants caused by soilborne pathogen that it is effective. The results suggest that considering the initial disease severity rating of 7.00 to 9.00 both *Trichoderma* treatments significantly suppressed the incidence of clubbing on cabbage roots at different levels but the application as basal is more effective.

Table 2. Disease control (%) of clubroot severity infection on cabbage.

Treatment	Mean
T_1 – Untreated	00.00
T_2 - *T. Koningii strain* Pure culture (in PDA)	57.92[b]
T_3 - *T. Koningii strain* in rice hull substrate	72.20[a]
CV %	24.25

Means from the same letter do not differ significantly at 5% level DMRT

Yield of Cabbage Heads

Table 3 presents the weight of cabbage heads. The highest weight of 26.33 tons/ha was obtained from *T. koningii* strain cultured in rice hull substrate applied as basal but comparable when applied as drench producing 20.03 tons/ha from the *T. koningii* strain pure culture. Numerically, however the application of *T. koningii* as basal produced heavier weights of cabbage heads. The lowest was 3.28 tons/ha. derived from the untreated plots. The differences in yield could be attributed to the clubroot disease severity as shown in Table 2. Cabbage heads produced from the untreated plots are relatively small, with loose leaf heads and others wilted and eventually die during the growing period.

Based from the study, the application of *t. koningii* can increase yield of cabbage to about 5 to 7 folds.

Table 3. Weight of marketable cabbage heads at harvest (tons/ha).

Treatment	Mean
T_1- Untreated	3.28^{c}
T_2 - *Trichoderma* Pure culture (in PDA)	20.03^{a}
T_3 - Trichoderma in rice hull substrate	26.33^{ab}
CV %	18.15

Means from the same letter do not differ significantly at 5% level DMRT

Study 3. ON-FARM EVALUATION OF T. *KONINGII* STRAIN AGAINST CLUBROOT DISEASE OF CRUCIFERS

RATIONALE

The use of biological control agents such as the *Trichoderma* has been demonstrated to combat soilborne pathogens. They have the capacity to adapt to different environmental conditions thus the microorganism can be utilized in different soil, crops climate and technological processes. It does not appear to be pathogenic to any plants, however it can parasitize, control and kill many fungi, nematode and phytopathogens that attack and destroy crop (Cooks and Baker, 1996).

The advantage of using biocontrol to combat plant diseases such as those present in the soil does not leave any residues hazardous to the environment and to

humans. Foremost is the environmental concern, risk and sustainability. Further it minimizes the use of chemicals because it does not only function for control of pests but also enhance the growth and development of plants which will increase the resistance or tolerance against pests attack (http://wjow.soilfertility.com/trichoderma /English/indexshtml).

OBJECTIVES

1. To verify the effectiveness of *T. koningii* strain cultured in rice hull substrate against clubroot disease in farmers field

2. To compare the effect of *T. koningii* strain and fungicide Pentachloronitrobenzene against clubroot disease of crucifers.

METHODOLOGY

The on-farm trial was conducted in the major crucifer growing farms severely infected with clubroot disease. The experiment were conducted in two sites situated in the Municipality of Buguias, Benguet in Barangay Loo and in sitio Bad-ayan. The farms were previously planted to heading lettuce and cabbage, respectively. Each farm had an area of about 300 sq m each.

The land was thoroughly prepared consisting of 7 plots each per treatment replicate measuring 1 x 5 sq. meters. Prior to planting, both farms were applied with chicken manure directly in the planting holes with rate of 4 tons per hectare. During hilling-up urea and triple -14 were applied in T_3 while only triple-14 was applied in T_2. Chinese cabbage variety WR 65 days was planted in sitio Bad-ayan while cabbage variety Scorpio was planted in Barangay Loo, respectively. The Chinese cabbage was directly seeded on the prepared mounds while the cabbage was transplanted 45 days after sowing. All cultural management practices were implemented such as irrigation, weeding and insecticide application against insect pest.

There were three treatments each replicated four times. The randomized complete block design was used in the study. The treatments were the following;

Treatment	Dosage
T_1 – Untreated	-
T_2 – *Trichoderma koningii strain* (cultured in rice hull substrate)	1 tbs/ planting hole
T_3 – Pentachloronitrobenzene	4 tbs/16 li water

The plots applied with the *Trichoderma* was mixed with chicken manure three weeks before transplanting while PCNB was tank mixed and applied directly in the planting holes one day before transplanting.

Data gathered:

1. **Initial and final soil pH.** Soil samples were collected at random before planting and during harvest. The soil was analyzed courtesy by the SAGREX Agrochemical Company.

2. **Disease severity (%).** Assessment was done by uprooting twenty matured cabbage and Chinese cabbage heads from each treatment replication. Roots were cut and washed. The scale used was the same as in study 2.

3. **Yield (tons/ha).** Weights of cabbage and Chinese cabbage heads were taken at harvest and computed in tons/hectare basis.

RESULTS AND DISCUSSION

Between the treatments, the application of *T. koningii* strain significantly suppressed clubroot infection and percent control on cabbage and Chinese cabbage with mean rating indices of 3.80 to 4.00 or major lateral root clubbed, 1-2 cm diameter to almost minor lateral root clubbing 0.50 cm diameter (Table 1). The application of PCNB had a higher mean score of 6.35 and 5.64 and moderate roots clubbed on tap root. The untreated had a disease a score of 8.55 to 9.00 or severe root clubbing with decayed roots.

Significantly, percentage disease control was higher from *T. koningii* of about 56% (cabbage) and 54% (Chinese cabbage) compared to PCNB with 30 and 28%, respectively.

The mechanism for the higher percentage control of the clubroot disease severity from the *Trichoderma* treatment could be due to a level of inhibition of the development of the pathogen by the beneficial microorganism.

Table 1. Disease severity and percent control of clubroot as affected by the treatments

Treatment	Disease severity			
	Loo		Bad-ayan	
	Rating disease	% control	Rating	% disease control
T_1 – Untreated	8.55[c]	0.0	9.00[c]	0.0
T_2 - *Trichoderma*	3.80[a]	56	4.10[a]	54
T_3 - Pentachlonitrobenzene	6.35[b]	30	6.64[b]	28
CV%	26.28		21.30	

Means from the same letter do not differ significantly at 5% level DMRT

On soil pH, the application of *Trichoderma* in both locations irrespective of the crop was raised to 6.50 (cabbage) and 5.80 (Chinese cabbage) from the initial of 4.0 and 4.08, respectively. The PCNB application has also raised the soil pH in both farms but very minimal from the initial of 4.0 and 4.08 to 4.70 to 4.80 at harvest which suggest that the soil is still acidic (Table 2).

According to the findings of Porth et al., (2004) and Howard et al., (2007) one of the most documented treatment for clubroot is raising the pH of soil with the application of *T. koningii* strain. They further claimed *T. koningii* strain acts soil conditioner. When the pH of soil is raised up to 7.2 or from very acidic to slightly acidy or alkaline, this create an unfavorable environment for the pathogen, most likely by disrupting the release of the zoospore thereby suppressing the severity of the disease paving the way for the availability of nutrient to be use by the plants.

Table 2. Soil pH before planting and after harvest

Treatments	Location			
	Loo, Buguias		Bad-ayan, Buguias	
	Cabbage		Chinese cabbage	
	Initial	final	Initial	final
T_1 - Untreated	4.00	4.30	3.88	4.10
T_2 – *Trichoderma koningii* strain	4.00	6.50	4.08	5.80
T_3 - Pentachlonitrobenzene	4.20	4.70	4.00	4.80

Table 3 presents the yield of the vegetable crops. Heavier weights of cabbage was obtained from the application of *Trichoderma* with 27 tons/hectare but comparable to 22.40 tons/hectare from PCNB treatment. Similarly, heavier weights of Chinese cabbage heads from the *Trichoderma* treatment but was comparable to PCNB with mean weights of 38.12 and 29.78 tons/hectare, respectively. The untreated plots planted to cabbage produced heads weighing 3.35 tons/ha and 6.70 tons/ha from Chinese cabbage.

The results show that there is a direct relationship of the clubroot disease severity to yield, the lower the infection rate the higher the weights of the crops and vice versa. This maybe due to the translocation of nutrients within the roots from the treated plots compared to untreated with severe clubroot infection hindering the passage of nutrients.

Table 3. Mean Weights marketable cabbage and Chinese cabbage heads (tons/ha)

Treatment	Cabbage	Chinese cabbage
T_1 - Untreated	3.45[c]	6.70[c]
T_2 - *Trichoderma* in rice hull substrate	27.95[a]	38.12[a]
T_3 - Pentachlonitrobenzene	22.40[ab]	29.78[ab]
CV (%)	18.30	24.20

Means from the same letter do not differ significantly at 5% level DMRT

Based from the economic analysis the use of *Trichoderma* against clubroot disease had a higher return on cash expense with 167% compared to PCNB with 164 %. The result is due to heavier weights of cabbage and Chinese cabbage heads in plots applied to *Trichoderma* (Table 4).

Table 4. Economic Analysis of cabbage

Cost of Production	Treatment		
	Untreated	*T. koningii* in rice hull	Pentachloronitrobenzene
Labor Cost	18355.44	18,355.44	18,355.44
Cost of Inputs	35136.00	57,771.00	44,166.00
Tools and Equipments	6500.00	6,500.00	6,500.00
TOTAL	59971.44	82,606.44	69,001.44
Gross Return	25,098.00	220,805.00	162,960.00
Net Income	(-34,872.69)	138,198.56	93,958.56
Return of Cash Expense (ROCE)		167.30%	136.17%

Table 5. Economic analysis of Chinese cabbage

Cost of Production	Treatment		
	Untreated	*Trichoderma* in rice hull	Pentachloronitrobenzene
Labor Cost	17,241.44	17,241.44	17,241.44
Cost of Inputs	36,002.00	59,097.00	43,122.00
Tools and Equipments	6,500.00	6,500.00	6,500.00
TOTAL	59,743.44	82,838.44	66,863.44
Gross Return	36,850.00	219,190.00	163,790.00
Net Income	(-22,893.44)	136,351.56	96,926.56
Return of Cash Expense (ROCE)		164.60%	144.96%

CONCLUSION

The rice hull is the most potent substrate for the culture of *Trichoderma koningii* strain under temperatures ranging from 21-22°C production the maximum spore count of 6.8 x10^7 in 30 days after inoculation under ambient condition and 10 days at temperatures ranging from 26 -30°C under plastic house. Although it ranked 4th in terms of profit (98.18%) it was the most favored among the six substrates tested.

On verification trial, the application of *T. koningii* strain grown in rice hull substrate applied as basal against clubroot disease of cabbage at 1tbs/ per planting hole mixed with chicken manure three weeks before planting markedly registered lower rating index of 2.62 equivalent to minor lateral root clubbed, 0.5 cm diameter. The application as soil drenched using a 10-day old spore colony applied 1 day before planting obtained a score of 4.41 or major lateral root clubbed 1-2 cm. diameters. The untreated had the highest with a score of 8.8 or severe roots clubbed.

On percentage control, *T. koningii* strain applied as basal significantly suppressed the disease infection compared when the same was applied as soil drenched with 72% and 57%, respectively. Further, yield obtained from the basal method produced heavier weights of marketable cabbage heads of 25.33 tons/ha and 19.00 tons/ha when applied as drenched. The yield of 3.28 tons per hectare was from the untreated.

Results from on-farm evaluation, *Trichoderma koningii* strain cultured in rice hull applied at 1tbs/ planting hole mixed with the chicken manure three weeks before planting was raised from very acidic to moderately acidic (4.0 and 4.08 to 6.50 and 5.90) in both experimental farms in Loo (cabbage) and Bad-ayan (Chinese cabbage) .The soil pH taken from fungicide PCNB was raised but it is considered still very acidic (4.20 and 4.00 to 4.70 and 4.80).

Significantly, disease severity of clubroot on cabbage and Chinese cabbage was suppressed by the *Trichoderma* treatment in both locations with rating indices of 3.80 and 4.10 or major lateral roots clubbed to almost minor lateral roots clubbed. The application of fungicide registered relatively higher clubroot infection but relatively lower compared to untreated plots with means of 6.35 and 5.64 and 8.55 to 9.00 or almost moderate clubbing of tap root and severe root clubbing and decaying of roots, respectively.

On percentage disease control, the application of *T. koningii* strain in rice hull obtained higher with 56% (cabbage) and 54% (Chinese cabbage) while 30% and 28% in that order from the application of fungicide PCNB.

Heavier weights of cabbage and Chinese cabbage heads was from the application of *T. koningii* strain with 27.95 and 38.12 tons/ha, respectively

which were comparable to the weights of 22.40 and 29tons/ha from PCNB. The lowest weights of 3.45 and 6.70 tons/hectare were from the untreated. Results reveals that the high weights of cabbage and Chinese cabbage from the *Trichoderma* treated plots could be attributed to the effect of the increase of soil pH from very acidic to moderately acidic making the nutrient available for the plants, and the lower severity of the clubroot disease leading to the translocation of nutrients from the root crops.

Recommendation

Based on the results of the study the following are recommended:

1. Rice hull could be used as alternative substrate in the mass production *Trichoderma* because it can produce the maximum spores in 30 days after spore inoculation at temperatures ranging from 21 – 22^{0}C under ambient condition. However, the maximum spores could be obtained in a shorter period at 10 days after inoculation when these are stored under temperatures ranging from 26 to 30^{0}C .
2. *Trichoderma* grown in rice hull applied as basal at 10 grams/per planting hole mixed with chicken manure three weeks before planting is recommended. Further, this method can suppress clubroot disease severity of crucifer crops by 54% up to 72%.

Future Plans

1. To continue mass production and distribution of the Trichoderma for farmers with clubroot disease infected area.
2. To continue testing the potential of the microorganism with other soil pathogen such bacterial wilt of potato, Fusarium of strawberry.

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ACKNOWLEDGEMENT

This is to acknowledge the seedpotato growers of Ganbang and Le-in, Bakun and BABODEMCO of Buguias for their support and providing their resources that made this endeavor successful .

APPENDICES

Table 1. Cost of Production of *Trichoderma* substrates (1 liter = 220 bags)

A. Operation Activity		
1. Preparation of media	1 mandays	
a. Sterilization		365.00
b. Isolation		
2. Preparation of substrate	2 mandays	
1. Packing		730.00
2. Sterilization		
3. Inoculation		
B. Cost of Inputs		
1. Agar (18gx 3)		54.00
2. Dextrose 10g x 3		30.00
3. Potato 15/kg		3.75
4. Plastic bags 220 x 3		660.00
5. Rubber band 220 x 0.17		37.50
6. Tissue		10.37
7. Denatured alcohol		25.00
8. Rubbing alcohol		16.25
9. Detergent		5.00
10.Match		0.50
11.Electricity		200.00
C. Tools and Equipment		
1. Inoculation chamber		3,000.00
2. Alcohol lamp		50.00
Total		**5,187.37**

Fine Rice Bran 6,276.37 = 72.6 kg x P15/kg = 1,089.00 + 5,187.37 =

Coarse Rice Bran 6,203.77 = 72.6 kg x P14/kg = 1,016.40 + 5,187.37 =

Rice Hull 5,332.57 = 72.6 kg x P 2/kg = 145.20 + 5,187.37 =

Mushroom Compost 5,5477.77 = 72.6 kg x P 4/kg = 290.40 + 5,187.37 =

Chicken Manure 5,405.17 = 72.6 kg x P 3/kg = 217.80 + 5 187.37 =

Mushroom Compost + 5,187.37 = 5,356.77
= 24.2 kg x P 4/kg
= 24.2 kg x P 3/kg
} = 96.8
= 72.6
169.4 +
Chicken Manure + Garden Soil (1:1:1)
= 24.2 kg

Price of *Trichoderma* Substrate = Php 50.00/bag x 220 bags = Php 11.000.00

Table 2. Economic analysis of *Trichoderma* substrate [1/]

Substrate	Total Cost of Production	Gross Return	Net Income	ROCE
Fine Rice Bran	6,276.37	11,000.00	4,723.63	75.26 %
Coarse Rice bran	6,203.77	11,000.00	4,796.23	77.31 %
Rice hull	5,332.57	11,000.00	5,667.43	106.28 %
Mushroom Compost	5,477.77	11,000.00	5,522.23	100.81%
Chicken Manure	5,405.17	11,000.00	5,594.83	103.51 %
Mushroom Compost + Chicken Manure + Garden Soil	5,356.37	11,000.00	5,643.63	105.36 %

[1/]Price of *Trichoderma* substrate/kg Ph 50.00 x 220 bags = Ph 11,000.00

Table 3. Cost of production of cabbage (One hectare)

I. COST OF PRODUCTION		
A. Labor Cost		
Operation/Activity	**Man – Hour****	**Value (P)**
1. Seedbed preparation	16	350.08
2. Sowing	10	218.80
3. Care of seedlings	20	437.60
4. Construction of plastic structure	20	437.60
5. Land preparation	144	3,150.72
6. Transplanting	68	1,487.84

7. Care and maintenance of plants	320	7,001.60
8. Harvesting, trimming and hauling	136	2,975.68
9. Grading and packing	104	2,275.52
Sub-total		**18,335.44**
B. Cost of Inputs		
T_1 - (UNTREATED)		
Item	**Quantity**	**Value (P)**
1. Seeds	200g @ 364/50g scorpio	1,456.00
2. Fertilizers	8 sacks T-14 @ 870/sack	6,960.00
3. Chicken manure	50 sacks @ 100/sack	5,000.00
4. Insecticides		16,200.00
5. Fungicides	8 kg @ 480/kg	3,840.00
6. Foliar Fertilizer	12 kg @ 300/kg	3,600.00
Sub-total		**35,136.00**

** Computed at P21.88/man-hour or P175/man-day

T_2 – *(Trichoderma)* in rice hull substrate		
1. Seeds	200g @ 364/50g scorpio	1,456.00
2. Fertilizers	4 sacks urea @ 740/sack	2960.00
3. Chicken manure	50 sacks @ 100/sack	5,000.00
4. Insecticides		11,640.00
5. Fungicides	8 kg @ 480/kg	3,840.00
6. *Trichoderma*	640 bags @ 50/bag	32,000.00
7. Application of *Trichoderma*	5 laborers x 175	875.00
Sub-total		**57,771.00**

T_3 – (Pentachloronitrobenzene) Drench		
1. Seeds	200g @ 364/50g scorpio	1,456.00
2. Fertilizers	8 sacksT-14@ 870/sack	6,960.00
3. Chicken manure	50 sacks @ 100/sack	5,000.00
4. Insecticides		16,200.00
5. Fungicides + PCNB	8 kg @ 480/kg + 16 kg @ 335/kg	9,200.00
6. Drenching of PCNB	10 laborers x 175/day	1,750.00
7. Foliar Fertilizer	12 kg @ 300/kg	3,600.00
Sub-total		**44,166.00**
C. Tools and Equipment		
1. 2 units knapsack sprayer	5,000.00	1,650.00
2. 5 pcs rake	975.00	325.00
3. 10 pcs light hoe	3,200.00	1,060.00

4. 5 pc shovel	2,440.00	810.00
5. 5 pc spading fork	2,500.00	825.00
6. 2 pc bolo	520.00	180.00
7. 2 pc sprinkler	770.00	260.00
8. 1 empty drum	700.00	235.00
9. 200 meters hose	2,000.00	495.00
10. Plastic sheet	1,500.00	495.00
Sub-total		**6,500.00**

Table 4. Gross Returns

T_1 – Untreated			
Class*	**Price**	**Yield (kg)**	**Amount**
A- 25%	9.50	862.50	8,193.75
B - 50%	8.00	1725.00	13,800.00
C -15%	6.00	517.50	3,105.00
Total			**25,098.75**
T_2 – Trichoderma in rice hull			
A – 60%	9.50	16,770	159,315.00
B - 20%	8.00	5,590.00	44,720.00
C – 10%	6.00	2,795.00	16,770.00
Total			**220,805.00**

T_3 – Pentachloronitrobenzene			
Class*	**Price**	**Yield (kg)**	**Amount**
A- 25%	9.50	5,600.00	53,200.00
B - 50%	8.00	11,200.00	89,600.00
C -15%	6.00	3,360.00	20,160.00
Total			**162,960.00**

* 10% provision for unmarketable heads, spoilage and price fluctuations

Table 5. Cost of Production in Chinese cabbage (One Hectare)

I. COST OF PRODUCTION		
A. Labor Cost		
Operation/Activity	**Man – Hour****	**Value (P)**
1. Seedbed preparation	16	350.08
2. Sowing	10	218.80
3. Care of seedlings	20	437.60
4. Construction of plastic structure	20	437.60
5. Land preparation	160	3,500.80
6. Transplanting	52	1137.76
7. Care and maintenance of plants	360	7,876.80
8. Harvesting	70	1,531.60

9. Grading and packing	80	1,750.40
Sub-total		**17,241.44**
** Computed at P21.88/man-hour or P175/man-day		
B. Cost of Inputs		
T_1 - (UNTREATED)		
Item	**Quantity**	**Value (P)**
1. Seeds	200g @ 348/50g	1,392.00
2. Fertilizers	11 sacks T-14 @ 870/sack	9,570.00
3. Chicken manure	50 sacks @ 100/sack	5,000.00
4. Insecticides		16,200.00
5. Fungicides	8 kg @ 480/kg	3,840.00
Sub-total		**36,002.00**

T_2 – (*Trichoderma*) in rice hull substrate		
1. Seeds	200g @ 348/50g	1,392.00
2. Fertilizers	5 sacks urea @ 740/sack	4,350.00
3. Chicken manure	50 sacks @ 100/sack	5,000.00
4. Insecticides		11,640.00
5. Fungicides	8 kg @ 480/kg	3,840.00
6. *Trichoderma*	640 bags @ 50/bag	32,000.00
7. Application of *Trichoderma*	5 laborers x 175	875.00
Sub-total		**59,097.00**

T_3 – (Pentachloronitrobenzene) Drench		
1. Seeds	200g @ 348/50g	1,392.00
2. Fertilizers	11 sacksT-14@ 870/sack	9,570.00
3. Chicken manure	50 sacks @ 100/sack	5,000.00
4. Insecticides		16,200.00
5. Fungicides + PCNB	8 kg @ 480/kg + 16 kg @ 335/kg	9,200.00
6. Drenching of PCNB	10 laborers x 175/day	1,750.00
Sub-total		**43,122.00**
C. Tools and Equipment		
1. 2 units knapsack sprayer	5,000.00	1,650.00
2. 5 pcs rake	975.00	325.00
3. 10 pcs light hoe	3,200.00	1,060.00
4. 5 pc shovel	2,440.00	810.00
5. 5 pc spading fork	2,500.00	825.00
6. 2 pc bolo	520.00	180.00
7. 2 pc sprinkler	770.00	260.00
8. 1 empty drum	700.00	235.00

9. 200 meters hose	2,000.00	495.00
10. Plastic sheet	1,500.00	495.00
Sub-total		**6,500.00**

Table 6. Gross Returns (Chinese cabbage)

	T_1 - Untreated		
Class*	**Price**	**Yield (kg)**	**Amount**
A- 25%	7.00	1,675.00	11,725.00
B - 50%	6.00	3,350.00	20,100.00
C -15%	5.00	1,005.00	5,025.00
Total			**36,850.00**
	T_2 – *Trichoderma* in rice hull		
A – 50%	7.00	19,060.00	133,420.00
B - 25%	6.00	9,530.00	57,180.00
C – 15%	5.00	5,718.00	28,590.00
Total			**219,190.00**

	T_3 - Pentachloronitrobenzene		
Class*	**Price**	**Yield (kg)**	**Amount**
A- 25%	7.00	7,445.00	52,155.00
B - 50%	6.00	14,890.00	89,340.00
C -15%	5.00	4,467.00	22,335.00
Total			**163,790.00**

* 10% provision for unmarketable heads, spoilage and price fluctuations

Table 7. Economic Analysis of Cabbage

Cost of Production	**Treatment**		
	Untreated	**Trichoderma in rice hull**	**Pentachloronitrobenzene**
Labor Cost	18,355.44	18,355.44	18,355.44
Cost of Inputs	35,136.00	57,771.00	44,166.00
Tools and Equipments	6,500.00	6,500.00	6,500.00
TOTAL	59,971.44	82,606.44	69,001.44
Gross Return	25,098.00	220,805.00	162,960.00
Net Income	(-34,872.69)	138,198.56	93,958.56
Return of Cash Expense (ROCE)		**167.30%**	**136.17%**

Table 8. Economic Analysis of Chinese cabbage

Cost of Production	Treatment		
	Untreated	*Trichoderma* in rice hull	Pentachloronitrobenzene
Labor Cost	17,241.44	17,241.44	17,241.44
Cost of Inputs	36,002.00	59,097.00	43,122.00
Tools and Equipments	6,500.00	6,500.00	6,500.00
TOTAL	59,743.44	82,838.44	66,863.44
Gross Return	36,850.00	219,190.00	163,790.00
Net Income	(-22,893.44)	136,351.56	96,926.56
Return of Cash Expense (ROCE)		164.60%	144.96%