BIBLIOGRAPHY

AGAPITO, EMERLOU P. APRIL 2012.Evaluation of Bacteria and Actinomycetes-like Organisms against Club Root *(PlasmodiophoraBrassicae*) in Chinese Cabbage *(BrassicaPekinensis)*. Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The study was conducted at the department of plant pathology green house, Benguet State University, LaTrinidad, Benguet from April 2011to January 2012to evaluate the effect of bacterial isolates and actinomycetes-like organismson the growth and development of Chinese cabbage and their effects on the incidence and severity of club root.

Seedling experiment revealed thatsix isolates from the dilution of 10^4, six isolates from the dilution of $10^5$and three isolates from the dilution of 10^6enhanced seed germination rate of chinese cabbage to 100%. Likewise, isolates 9 and 10sigificantly improved the height of seedlings using dilutions 10^4,10^5and10^6.

On the other hand, pot experiment showed that isolates 3, 4, 5,6,1 and 9 significantly increased the nitrogen content of the soil after harvest. In addition, isolates 10, 3 and 9 gave the lowest percent club root infection of 26.66 %, 40 % and 46.67%. The same isolates gave the lowest club root severity infection of 2.3 % and 2.67 %. In terms of vegetative fresh weight, isolate 1 gave the highest.

From the results, some bacterial isolates and actinomycetes-like organism from sunflower soil improved seed germination rate and plant height, enhanced nitrogen uptake and reduced club root severity.

TABLE OF CONTENTS

INTRODUCTION

Non adverse effects on the environment of biocontrol strategies of pest management are priorities of tomorrow's world agriculture (Baniasadi, 2009). Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and the ecosystem. It neither eliminates the pathogen nor the disease, but brings them into natural balance. Intensive research on plant growth promoting bacteria (PGPB) is underway worldwide for developing biofertilizers and biocontrol agents (BCAs) as better alternatives to chemicals (Ningthoujam, 2009). High input agriculture is increasingly recognized as contributing to the degradation of environment and health besides demanding high costs due to its dependence on chemical inputs (Sulastri, 2005).

Biocontrol with beneficial bacteria is one promising alternative to fungicides. Hydrolases such as chitinase contribute to degradation of fungal cell walls. Chitin is the second most abundant polysaccharide in nature and a major component of fungal walls, insect exoskeletons and crustacean shells. Chitinase secreted by biological control agents is likely to be effective against pathogenic fungi which cell walls are mainly made up of chitin (Ningthoujam, 2009).Actinomycetes are active biocontrol agents due to their antagonistic properties against wide range of plant pathogenic fungi (Baniasadi, 2009). This group of microorganisms is best known for their ability to produce bio-active metabolites including antibiotics, plant growth factors, and other substances. Many of the presently used antibiotics such as streptomycin, gentamicin, rifamycin and erythromycin are the product of Actinomycetes (Jeffrey, 2008).

Among Actinomycetes, the Streptomycetes are the dominant. The non-streptomycetes are called rare Actinomycetes, comprising approximately 100 genera (Sivakumar, 2008). Streptomycesand other Actionmycetes are major contributors to biological buffering of soils and have roles in organic matter decomposition conducive to crop production.

Actinomycetesare responsible for much of the digestion of resistant carbohydrates such as chitin and cellulose bioremediation. The number and types of Actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. Some are able to grow at elevated temperatures (>50°C) and are essential to the composting process (Burge, 2008).Population ofActinomycetes is relatively lower than other soil microbes and contains a predominance of Streptomyces that are tolerant to acid conditions. Arid soils of alkaline pH tend to contain fewer Streptomyces and more of the rare genera such as Actinoplanes and Streptosporangium. However, alkaliphilicActionmycetes will provide a valuable resource for novel products of industrial interest, including enzymes and antimicrobial agents. Among Actinomycetes, the Streptomyces are especially prolific. A search of the recent literature revealed that at least 4,607 patents have been issued on Actinomycete related product and processes. *Streptomyces*covers around 80% of total antibiotic product, with other genera trailing numerically. Micromonospora is second with less than one-tenth as many as Streptomyces(Arifuzzaman, 2010).

The result that will be generated in this study whereby a new bacterial isolates that will be found effective as an enhancer of nutrients in the soil will contribute for the growth and development of plants. Moreover the isolates that will be found effective as biological agent against club root will be added to the few existing bio control agents used to manage the disease.

This study aimed to:

1. determine the effects of bacteria and Actinomycetes-like organisms on the growth and development of chinese cabbage, and
2. determine their effects on the incidence and development of club root on Chinese cabbage.

This study was conducted at the department of plant pathology green house, College of Agriculture, Benguet State University, La Trinidad, Benguet from April 2011 to January 2012.

REVIEW OF LITERATURE

The Nomenclatureand Classification
of theActinomycetes

Family *Streptomycetaceae.*Actinomycetes with branched slender mycelium that is rarely or not septate forming spores on aerial hyphae and not fragmenting intooidia. There are two genera, Streptomyces and Micromonospora(Waksman and Henrici, 1943)

Genus *Streptomyces.Streptomycetaceae* forming spores in chains on aerialhyphae called sporosphores. Sporosphores are apparently endogenous in origin, formed by a segregationof protoplasm within the hypha into a series of round, oval or cylindric bodies.Chains of spores are often spirally coiled. Sporophores may be simple orbranched. The type of species of this newly-named genus is *Streptomyces albus (Rossi-Doria emend Krainsky) comb. nov*. This species was formerly known as *ActinomycesalbusKrainsky* and first described as *StreptothrixalbaRossi-Doria*. This is one of the most common and best known species of the group. It is colorless with white aerial mycelium, forming ovoidal spores in coiled chains on lateral branches of the aerial hyphae. It is proteolytic, liquefying gelatin and peptonizing milk with the production of alkaline reaction in the latter. It does not produce any soluble pigment eitheron an organic or synthetic medium, but does produce a characteristic earthy ormusty odor (Waksman and Henrici, 1943).

Genus *Micromonospora*.The name *MicromonosporaOrskov*, apply to those forms which producing single conidia on lateral branches. Tsiklinskyhad previously applied the name *Thermoactinomyces* to species of thisgroup, whose identity is clear from photomicrographs. But in her descriptionof the genus she also included thermophilic species with catenulate spores, basingthe genus on temperature relations rather on morphology (Waksman, 1959).

Isolation, Identification, Cultivation, and Preservation

Most of the techniques used in the isolation and cultivation of bacteria and fungi also apply to Actinomycetes. The isolation of these organisms from soils and other natural substrates is brought about by first plating out such materials in proper dilutions on suitable agar or gelatin media. The plates are incubated at favorable temperatures, for 2 to 7 days, and the colonies picked and transferred to sterile liquid or solid media for further development. A colony of an Actinomycetediffers from a bacterial colony due to the presence of a filamentous extension of the original cell or cells, spores, and degradation products. It is not an accumulation of cells originating from one or more similar cells. These are compact, often leathery, giving a conical appearance, and have a drysurface. According to Titus and Pereira (2005) the leathery or powdery appearance of Actinomycetes colonies is due to the production of conidia. These are often covered with aerial mycelium. When grown in liquid culture, either in a stationary or in a submerged condition, the majority of Actinomycetes, notably members of the genera *Streptomyces* and *Micromonospora*, grow in the form of flakes or spherical compact masses, leaving the medium clear. The mass of growth can easily be removed by filtration through

ordinary paper. Only when growth undergoes lysis do the cellsdisintegrate completely and a certain degree of turbidity occurs (Waksman, 1959).

The methods of studying the Actinomycetes population of soil, water, compost, and other materials include microscopic observations,plate culture studies,and selective culture procedures. Primarily for characterization and identification purposes,a standard media, comprising both synthetic and organic, are most essential. Synthetic, chiefly inorganic, media have found extensive application in the study of the morphology, physiology, and cultural characterization of these organisms. Organic media are used for obtaining supplementary evidence of a cultural nature, especially for strains that do not grow at all or grow only very quickly on the common inorganic media. A Media used primarily for obtaining maximum growth, especially for the maximum production of certain chemical substances, such as antibiotics, vitamins, or enzymes are usually complex in composition, utilizing plant and animal materials directly or after preliminary enzymatic or acid digestion. For maintaining cultures of Actinomycetes in such a manner as to reduce, to a minimum, degeneration and variationof the culture a suitable media, comprising both artificial and natural, such as sterile soil, and suitable conditions of growth thus make possible for the preservation of type cultures for comparative purposes. The great majority of Actinomycetes are aerobic and very few are anaerobic and many are microaerophilic. To supply proper aeration, the organisms are grown on the surface of solid media, or in shallow liquid layers, or in a thoroughly aerated submerged condition. For anaerobic growth, special procedures are required. Temperatures of 25-30° C are usually used for incubation of the great majority of *Steptomyces*, *Nocardias*, and

Micromonosporas. Pathogenic organisms require 37° C, and thermopiles usually require 50-60° C (Waksman, 1959).

Nitrogen Fixation

Various reports have been made in the past of the ability of one or more Actinomycetes to fix atmospheric nitrogen. Meyen first observed nodules on alder roots whichwas confirmed by Woronin. Brunchorst named the microbe microbes inside the nodules*Frankiasubtilis* and Hiltner recognized the nodule inhabitant as an actinomycete, gram-positive bacteria closely related to *Streptomyces*. Like *Streptomyces*, *Frankia*forms spores, but it also produces structures known as vesiclesthat sequester the oxygen-labile enzyme nitrogenase. The vesicle cell walls are composed ofhopanoid lipids, making them impervious to oxygen. Itappears phase-bright under phasecontrast microscopy. Several different *Frankia*strains were alsoisolated from actinorhizal plants, including *Casuarina*, *Elaeagnus*,and *Myrica*. Other Actinomycetes were also being isolated from the nodules of diverse actinorhizalplants, but not much attention was being paid to them. In the late 1980's, several Actinomyceteshad been isolated from nodules of *Casuarina*trees (indigenous to Australia) growing in Mexico (Hirsch, 2009).

twonocardias, *N. calcarca* and *N. cdlulans*, isolated from grassland lime soils were found to have the capacity to fix atmospheric nitrogen to the extent of 2.0 to 4.5 mg of X/gm of glucose or other carbon source in the medium. The second culture was also capable of decomposing cellulose, the amount of nitrogen fixed being 5 to 12 mg of X/gm of cellulose decomposed (Waksman, 1959).

Activity of Actinomycetes upon Plant Pathogenic Fungi

An extensive literature has accumulated upon the antagonistic effects of Actinomycetes upon fungi, especially upon plant pathogens. Winterpresented further evidence concerning the ability of various Actinomycetes to attack *Ophiobolusgraminus*, an important parasite that attacks wheat. Sanford and Cormack tested the effect of eight cultures of Actinomycetes upon the disease-producing fungus *Helminthosporiumsativum*. In comparison with a disease rating of 66 percent for the untreated pathogen, four Actinomycetes suppressed the virulence of the pathogen to 33, 22, and 1 per cent, respectively; two had no marked effect; and the other two appeared to increase the virulence by 12 and 16 percent, respectively. Perrault demonstrated that the growth of *Colletotrichumsepedonicum* in agar media was impeded by several microorganisms isolated from potato tubers affected with ring rot. Four of these organisms were Actinomycetes and were able to produce antibiotic substances that diffused readily through the medium and prevented all growth of the pathogen. One culture produced a lysis of the plant pathogen (Waksman, 1959). In 2005 plant pathology journal, 10 isolates of Actinomycetes was reported to have an antagonistic reaction to a single isolate of *Alternariasolani* through agar plate method (ShahidiBonjar, 2005).

Causation of Plant Diseases

In spite of the great importance of Actinomycetes in nature, especially in the soil, the number of plants attacked by these, as compared to the number of plants attacked by bacteria, fungi, and viruses, is rather limited. Two species of plant which is the Irish potato and the sugar beet plants are known to be infected by Actinomycetes and causes

scab. In Hoffmann's work, numerous infection experiments were carried out with twenty *Streptomyces*species, using a number of scab-susceptible potato varieties in the greenhouse and under field conditions. He found out that*S. scabies* was the pathogen of potato scab and the same was true with beet scab (Waksman, 1959).

Plant growth Promotion Activity
of Secondary Metabolites

Although Actinomycetes produce numerous kinds of secondary metabolites, their plant bioactivity is known very little. In their recent studies,Igarashi *et al*. (2006) have identified at least 10 chemically different classes of secondary metabolites produced by *Streptomyces hygroscopicus*. Of these compounds, pteridic acid A induced the adventitious root formation of kidney bean hypocotyls and growth promotion of tobacco BY-2 cells which suggest the possible involvement of secondary metabolites in plant growth promotion.

MATERIALS AND METHOD

Sterilized forest soil obtained at the forest area of Long long, Puguis, la Trinidad, Benguet was used in the entire experiment.The sterilization period using steam pressured drum lasted for 8 hours.

Seedling Experiment

Sterilized soil was distributed in a seedling tray with 3 x 13 holes. Different dilutions of 10^4, 10^5 and 10^6 of the different isolates were prepared and 3ml each was inoculated in seedling trays.The control was inoculated with water only. The experiment set up followed the Complete Randomized Factorial Design (CRD Factorial) and was replicated four times with 34 sample plants per treatment per replicate. Chinese cabbage seeds were sown two weeks after inoculation. The treatments are shown below.

Treatments:

Factor A - Isolates

T_0 -no bio-control added

Factor B – Dilution Rates

T_1 - bacterial isolate 1

T_2 - bacterial isolate 2

T_3- bacterial isolate 3

T_4- bacterial isolate 4

T_5 - bacterial isolate 5

T_6 - bacterial isolate 6

T_7 -bacterial isolate 7

T_8-bacterial isolate 8

T_9-bacterial isolate 9

T_{10}-bacterial isolate 10

Pot Experiment

The dilution of the different isolates that showed good performance during the seedling experiment was further evaluated in a pot experiment. An amount of six kg of soil were filled in pots measuring 6x6x11 inches and were inoculated with 30 ml 10^5 (best dilution) of the different isolates. Two weeks after the introduction of bacterial isolates, every pot was infested with 5ml*Plasmodiophorabrassicae*having sporeconcentration of $1x10^6$ per ml.Seedlings that were taken from the seedling experiment were transplanted in the inoculated pots one week after infestation. The experiment set up utilized the complete randomized design (CRD) with 3 replicates having 5 sample plants per treatment per replicate. The treatments are described below while figure 1 shows the isolates used:

Treatments:

T_0 -no bio-control added

T_1 - bacterial isolate 1

T_2 - bacterial isolate 2

T_3- bacterial isolate 3

T_4- bacterial isolate 4

T_5 - bacterial isolate 5

T_6 - bacterial isolate 6

T_7 -bacterial isolate 7

T_8-bacterial isolate 8

T_9-bacterial isolate 9

T_{10}-bacterial isolate 10

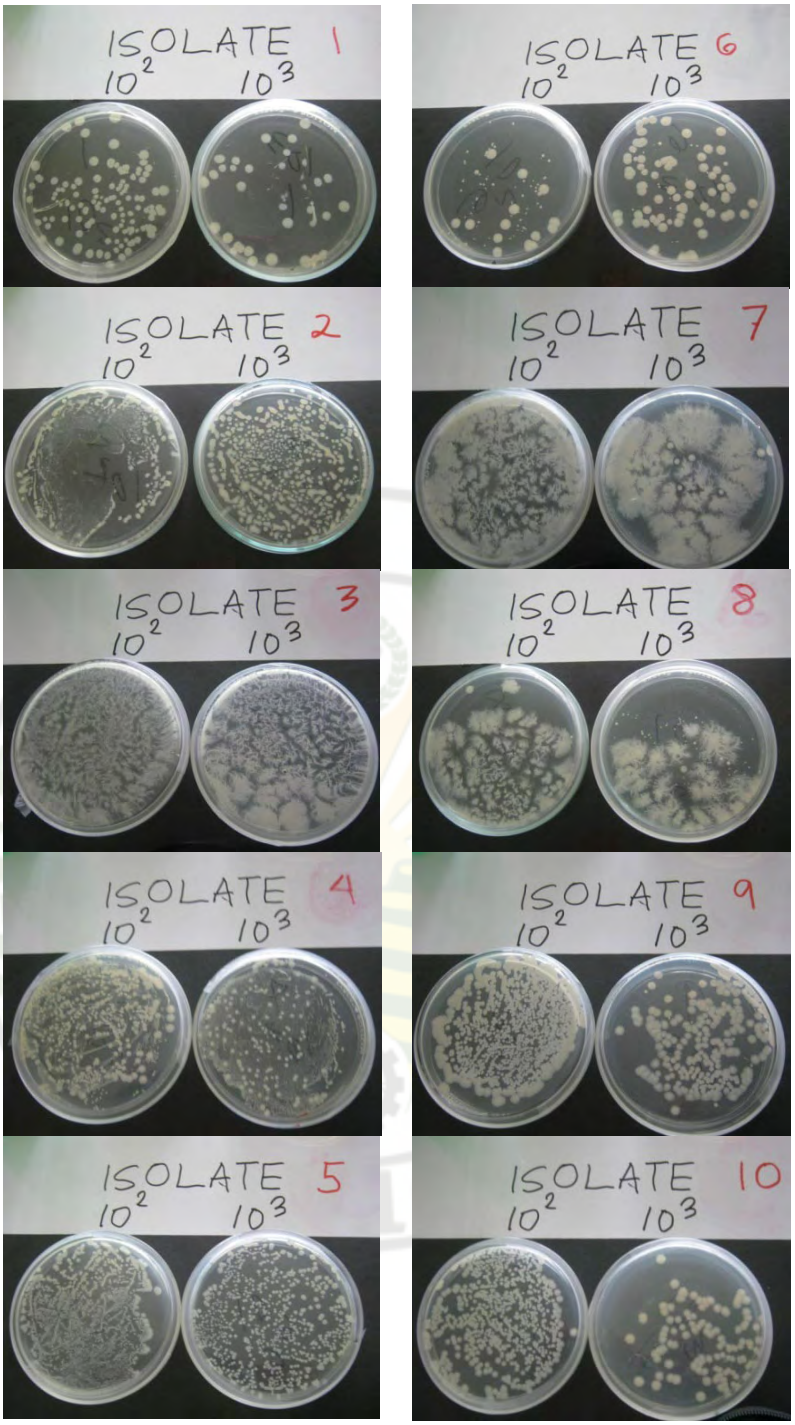

Figure 1. Colonies of the bacterial isolates used in the seedling and pot experiment

Data Gathered:

A. Seedling experiment

1. Percentage seed germination. Germinated seeds was counted two weeks aftersowing. Percent germination was determined using the formula,

$$\frac{\text{no.of germinated seeds}}{\text{no.of seeds sown}} \text{ x } 100$$

2. Seedlingheight. Height (inches) of all the 34 samples seedlings per replicate were measured after six weeks.

3. Seedling root length. Roots of three sample plants were uprooted randomly were measured.

B. Pot experiment

1. Nitrogen analysis of the soil. Soil samples of each replicate of the different treatment weighing 200 grams was collected after which was mix to come up with a composite soil sample. The percentage N content(%N) of the soil wasestimated by determining the organic matter content (%OM) of the soil multiplied by 0.05(%N= %OM x 0.05). Soil analysis was done before the inoculation of bacterial isolateand after harvest.

2. Final Height. Plant height of five samples was measured in inches one day before theharvest.

3. Club Root Incidence. Plants infected with of club root was counted at harvest and percent club root incidence was determined using the formula:

$$\% \text{ Incidence} = \frac{\text{no.of sample plants} - \text{no.of healthy plants}}{\text{total no.of plants}} \text{x } 100$$

4. Club root Severity. Assessment of club root severity was based on the rating scale of Anderson *et al*. as stated below:

Rating	Description
1	Normal root
2	Minor lateral clubbing at 0.5 cm diameter
3	Minor lateral clubbing at 1.2 cm diameter
4	Moderate clubbing
5	Severe clubbing in the tap root
6	Root decaying due advance infestation with plant death.

5. Fresh and dry weights of vegetative parts. Fresh weights in grams of the vegetative part was recorded before drying, whilethe dry weight was taken seven weeks after air drying.

6. Fresh and dry weights of below ground parts. Fresh weights in grams of the below ground partswas recorded before drying, while the dry weight was taken seven weeks after air drying.

RESULTS AND DISCUSSION

A. Seedling Experiment

Percentage Seed Germination
asAffectedby the Different Isolates

According to Lindgren (1992), the standard percentage seed germination in chinese cabbage is 80 % under optimum temperature.Table 1showsthe result of germination rate two weeks after sowing.

The highest germination rate of 100% was recorded under isolate 4.Isolate 1 provided the second highest germination rate of 98.95% followed by isolates 2, 7 and 9. Such result indicates that these isolates can be used as soil inoculants to improve seed germination for they gave a better rate of germination than the control (96.86 %)

Percentage Seed Germination
asAffectedby the Different Dilution Rates

The highest germination rate of 98.12% and 97.18 % was recorded from the dilutions of $10^4$and 10^5.This means that higher concentration of isolates work effectively in improving the germination rate of Chinese cabbage.

Interaction Effect on the Seed
Germination

Interaction between the factors suggest that all isolateimproved the germination rate of chinese cabbage using dilutions of $10^4$and 10^5 when inoculated in the soil before sowing the seeds.Isolates withthe dilution of 10^6hadthe least percentage germination.

Table 1. Percentage germination as affected by the different isolates and the differentdilution ratesrecorded after two weeks

TREATMENTS	DILUTION RATE			MEAN
	10^4	10^5	10^6	
Control	N/A	N/A	N/A	96.86%
Isolate 1	100%	100%	96.86%	98.95%
Isolate 2	100%	100%	93.75%	97.92%
Isolate 3	100%	90.66%	100%	96.89%
Isolate 4	100%	100%	100%	100%
Isolate 5	90.66%	100%	96.86%	95.84%
Isolate 6	96.86%	96.86%	93.73%	95.82%
Isolate 7	100%	100%	93.75%	97.92%
Isolate 8	96.86%	87.5%	96.86%	93.74%
Isolate 9	96.86%	96.86%	100%	97.91%
Isolate 10	100%	100%	87%	95.67%
MEAN	98.124%	97.188%	95.881%	

SeedlingsHeight as Affected bythe Different Isolates

In terms of seedling height, Table 2 revealed that soils inoculated with isolate10 gave the tallest seedlings with the average height of 13.82 inches. This wasfollowed by sample plantsunder isolates 3,9 and 4surpassing the control which means that thesecan significantly enhancethe seedling height of chinese cabbage. Isolates 7, 1, and 6 slightly increase the height of chinese cabbage.

Seedlings Height as Affected
bythe Different Dilution Rates

statistical analysis for the effect of different dilution rates (Table 2) shows that the best dilutions that that can enhance the seedling height were $10^5$and 10^6.

Interaction Effect on the Seedlings Height

Isolates 10, 3, 9 and 4 significantly improved the growth of chinese cabbage seedlings using any of the dilutions while. Seedlings under isolate 10 with the dilution of 10^5 had the highest measurement of height.

Table 2: Seedling height (inches) as affected by the different isolates and the different dilution rates measured after six weeks

TREATMENTS	DILUTION RATE			MEAN
	10^4	10^5	10^6	
Control	N/A	N/A	N/A	2.51
Isolate 1	2.99	4.01	3.79	3.60^{d}
Isolate 2	2.86	3.23	3.55	3.21^{d}
Isolate 3	3.98	4.26	4.25	4.16^{b}
Isolate 4	4.98	3.73	3.32	4.01^{d}
Isolate 5	2.71	3.28	3.53	3.17^{d}
Isolate 6	3.11	4.01	3.66	3.59^{cd}
Isolate 7	3.17	3.72	3.99	3.63^{d}
Isolate 8	2.96	3.53	3.29	3.26^{d}
Isolate 9	3.63	4.42	4.20	4.08^{bc}
Isolate 10	4.15	5.01	4.66	4.61^{a}
MEAN	3.454^{b}	3.92^{a}	3.824^{a}	

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

Figures 2 to 12 show the different set-up inoculated with isolates at different dilution rate.

Figure 2. Seedlings not inoculated with bacterial isolates (water)

Figure 3. Chinese cabbage seedlings inoculated with bacterial isolate 1

Figure 4. Chinese cabbage seedlings inoculated with bacterial isolate 2

Figure 5. Chinese cabbage seedlings inoculated with bacterial isolate 3

ure 6. Chinese cabbage seedlings inoculated with bacterial isolate 4

Figure 7. Chinese cabbage seedlings inoculated with bacterial isolate 5

Figure 8. Chinese cabbage seedlings inoculated with bacterial isolate 6

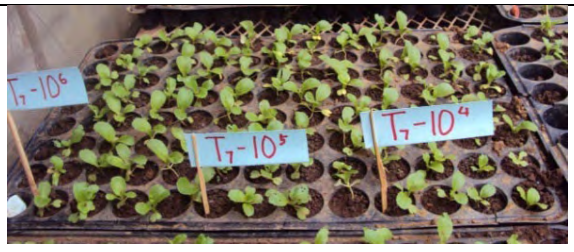

Figure 9. Chinese cabbage seedlings inoculated with bacterial isolate 7

Figure 10. Chinese cabbage seedlings inoculated with bacterial isolate 8

Figure 11. Chinese cabbage seedlings inoculated with bacterial isolate 9

Figure 12. Chinese cabbage seedlings inoculated with bacterial isolate 10

SeedlingsRoot Length as Affected Bythe Different Isolates

Table 3shows that all isolates enhanced the root length of chinese cabbage seedling by surpassing the control. Isolate 6provided the highest root length mean of 11.37followed by isolate 5.

SeedlingsRoot Length as Affected by the Different Dilution Rates

Result obtained for the effect of different dilutions on the root length of chinese cabbage is almost the same which suggest that all dilution rates has no significant effect on the root development of chinese cabbage.

Interaction Effect on the Seedlings
Root Length

The interaction between two factors suggests that isolate 6 with the dilution of 10^5will improve the root development of roots in terms of length giving the highest mean of 11.64. Main while dilution of 10^5used for isolate 7 gave the lowest mean of 8.25.

Table 3.Root length (inches) as affected by the different isolates and the different dilution rates measured after six weeks

TREATMENTS	DILUTION RATE			MEAN
	10^4	10^5	10^6	
Control	N/A	N/A	N/A	07.62
Isolate 1	09.91	10.23	08.43	09.52
Isolate 2	10.15	09.73	10.89	10.26
Isolate 3	08.28	09.57	09.80	09.22
Isolate 4	10.78	11.06	10.98	10.94
Isolate 5	11.04	10.44	11.59	11.02
Isolate 6	10.68	11.64	11.80	11.37
Isolate 7	09.71	08.25	10.69	09.55
Isolate 8	10.86	11.48	10.23	10.86
Isolate 9	10.78	11.38	10.46	10.87
Isolate 10	11.04	09.54	11.37	10.65
MEAN	10.32	10.33	10.62	

B. Pot Experiment

Based on the result of seedling experiment, all isolates has a good effect on the growth and development of chinese cabbage seedling. Using different rate of dilutions, isolates with the dilution of 10^5 showed the most significantresult. This served as the standard dilution rate for all isolate that was evaluated in the pot experiment.

Nitrogen Content of the Soil

Based on the result of % N content analysis as shown in Table 4, nitrogen content all soil samples that were inoculated with bacterial isolatehad increased. Initial soils withanitrogen content of 0.23 %,had significantlyincreased to0.34 %with the inoculation of isolate 3. On the other hand, isolate 8 slightly increase the amount of nitrogen in the soil.Under control, no significant increase can be observed.

Table 4.Initial and final percentage nitrogen content of soil samples, taken before the inoculation of bacterial isolatesand after harvest

TREATMENTS	REPLICATES			TOTAL	MEAN
	R_1	R_2	R_3		
Initial soil	0.23	0.25	0.22	0.70	0.23^{c}
Control	0.25	0.24	0.23	0.72	0.24^{bc}
Isolate 1	0.30	0.32	0.31	0.93	0.31^{a}
Isolate 2	0.30	0.31	0.30	0.91	0.30^{a}
Isolate 3	0.29	0.37	0.35	1.01	0.34^{a*}
Isolate 4	0.30	0.39	0.29	0.98	0.33^{a}
Isolate 5	0.30	0.37	0.31	0.98	0.33^{a}
Isolate 6	0.32	0.35	0.26	0.93	0.31^{a}
Isolate 7	0.31	0.32	0.24	0.87	0.29^{ab}
Isolate 8	0.30	0.31	0.23	0.84	0.28^{abc}
Isolate 9	0.29	0.30	0.32	0.91	0.30^{a}
Isolate 10	0.27	0.33	0.26	0.86	0.29^{ab}

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

Final Height

As table 5 shows, Isolate 8 enhanced the height of Chinese cabbagehowever isolates2, 1, 7, 5, and 9 slightly gave different result with the control. Isolate3gave a similar result with the controlwhereasisolate 6, 5 and 4 did not improve the height of chinese cabbage as less result was obtained compared to the control.

Percentage Incidence of Club Root

Table 6 shows that significantnumber of club root infectionappeared under the control followed by isolate 6, isolate 4 and 5, isolate 1 and 7, isolate 2, isolate 8, isolate 9 and isolate 3 then isolate 10 as the lowest. Low number of infection from isolate 10, 3 and 9 implies that the bacterial isolates inoculated in the soilcan lessen club root infection compared other isolates. These isolates can be a good bio control agent against club root.

Table 5.Effect of the different Isolates on the final height (inches)of Chinese cabbage taken after three months

TREATMENT	MEAN	
	ACTUAL	TRANSFORMED
control	6.97	2.73
isolate 1	7.87	2.89
isolate 2	8.20	2.95
isolate 3	6.97	2.73
isolate 4	4.67	2.06
isolate 5	7.13	2.76
isolate 6	5.80	2.26
isolate 7	7.63	2.85
isolate 8	8.67	3.01
isolate 9	7.40	2.80
isolate 10	7.13	2.76

Table 6.Percentage (%) incidence of club root as affected by the different isolates taken at harvest

TREATMENTS	REPLICATES			TOTAL	MEAN
	R_1	R_2	R_3		
control	100%	100%	80%	280%	93.33%
isolate 1	60%	60%	100%	220%	73.33%
isolate 2	100%	60%	40%	200%	66.67%
isolate 3	40%	40%	40%	120%	40.00%
isolate 4	100%	80%	60%	240%	80.00%
isolate 5	80%	80%	80%	240%	80.00%
isolate 6	80%	80%	100%	260%	86.67%
isolate 7	60%	80%	80%	220%	73.33%
isolate 8	20%	100%	60%	180%	60.00%
isolate 9	40%	40%	60%	140%	46.67%
isolate 10	20%	40%	20%	80%	26.66%

Club Root Severity

Result of club root severity (Table 7) corresponds with the result of club root incidence which means that samples with the highest incidence also has the highest severity. Sample plants under the control showed the highest club root severity rating followed by sample plants under isolate 4, 5 and 6, then isolates 1, 2 and 7, isolates 8, 3 and 9 then lowest severity is recorded under isolate 10. Reduction of club root severity in treatments 10, 9 and 3 suggests that these bacterial isolates applied to the soil can suppress club root development.

Table 7. Club root severity as affected by the different isolates, taken at harvest

TREATMENTS	REPLICATIONS			TOTAL	MEAN
	R_1	R_2	R_3		
Control	6	5	5	16	5.33[a]
Isolate 1	3	4	5	12	4.00[abc]
Isolate 2	5	4	2	11	3.67[abc]
Isolate 3	3	3	2	08	2.67[c]
Isolate 4	6	4	4	14	4.67[ab]
Isolate 5	5	5	4	14	4.67[ab]
Isolate 6	4	4	6	14	4.67[ab]
Isolate 7	3	4	4	11	3.67[abc]
Isolate 8	2	4	3	09	3.00[bc]
Isolate 9	3	3	2	08	2.67[c]
Isolate 10	2	3	2	07	2.33[c]

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

Fresh Weight of Vegetitave Parts

Among all isolates,isolate 1provides the highest weight (Table 8). Since better result was obtained compared with control, the result implies that isolate 1 can enhanced the vegetation of c. cabbage. Isolate 8 is the second highest followed by isolate 2, 3 then7. Isolate 4 has the lowest weight as maybe caused by the early death of some sample plants due to severe club root infection.Figure 1 shows the sample plant from treatment 4 that was severely infected with club root.

Figure 13. Sample plant severely infected by club root

Table 8.Fresh weights of vegetitave parts (g) taken at harvest

TREATMENT	MEAN	
	ACTUAL	TRANSFORMED
Control	27.33	5.07^{bc}
Isolate 1	100.0	9.99^{a}
Isolate 2	61.00	7.72^{abc}
Isolate 3	57.67	7.50^{abc}
Isolate 4	17.00	3.62^{c}
Isolate 5	32.67	5.66^{abc}
Isolate 6	50.00	6.03^{abc}
Isolate 7	53.67	7.35^{abc}
Isolate 8	73.67	8.23^{ab}
Isolate 9	50.67	6.84^{abc}
Isolate 10	44.67	6.71^{abc}

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

Dry Weight of Vegetitave Parts (g)

The aerial dry weight of Chinese cabbage was very significant under isolate1 (Table 9). This result shows that isolate 1 is a good plant growth promoting bacteria since it gave the highest dry weight. Isolate2, 3, and 7 and isolate 8gives result that is similar with the control. Whereas isolate4, 5, 6, 9 and 10provide lower weight that was obtained under the control.

Other Observations

Severe attack (Figure 14 and 15) of insects during the conduct of the experiment affected the vegetative part of the plants including the quality of the harvest. Incidence of cabbage butterfly larvae was high at the start of the study. Cultural practice was implemented however at the later part of the study, severe attack of diamond back moth larvae occurred. The use of integrated pest management approach was applied but found to be ineffective because of severe insect infestation. Use of organic approach was not part of the option since it might interfere on the effects of the isolates.

Table 9.Dry weightof vegetative parts (g),obtained seven weeksafter air drying

TREATMENT	MEAN	
	ACTUAL	TRANSFORMED
Control	2.79	1.81^{ab}
Isolate 1	5.21	2.38^{a}
Isolate 2	2.93	1.84^{ab}
Isolate 3	2.62	1.74^{ab}
Isolate 4	0.75	1.25^{b}
Isolate 5	1.52	1.43^{b}
Isolate 6	1.98	1.48^{b}
Isolate 7	3.21	1.93^{ab}
Isolate 8	3.16	1.89^{ab}
Isolate 9	2.12	1.60^{b}
Isolate 10	1.57	1.41^{b}

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

Figure 14.Chinese cabbage sample plant infestedby cabbage butterfly larvae

Figure 15. Skeletonized chinese cabbage infested bydiamond back moth larvae

Fresh Root Weight

Statistical analysis revealed that no isolates had significant effect on the fresh root weight of Chinese cabbage. However based on the actual data, highest weight was obtained in the control (Table 10). Comparison of root weight with the club root severity implies that club root severity influence root weight. The relationship between the club root and root weight states that the higher the club root severity of roots, the higher is the tendency of the root to gain more weight due to hypertrophy.

Table 10.Fresh weight of roots (g) taken at harvest

TREATMENT	MEAN	
	ACTUAL	TRANSFORMED
control	2.77	1.74
isolate 1	1.30	1.33
isolate 2	2.63	1.76
isolate 3	0.66	1.07
isolate 4	0.85	1.10
isolate 5	1.43	1.32
isolate 6	0.74	1.07
isolate 7	1.07	1.23
isolate 8	1.83	1.44
isolate 9	0.69	1.08
isolate 10	0.75	1.14

Root Dry Weight

Inoculated chinese cabbage with isolate 2 (Table 11) gave the highestdry root weight and is significantly different with plants inoculated with isolate 5, 6, 3 and 4. This observation is consistent with the result of fresh root weight. Isolate 10 which has the lowest severity rating also has direct relationship with the root dry weight. The relationship states thatthe lower the club root severity is, the lesser the weight of the roots will be.

Table 11.Roots dry weight (g) recorded after seven weeks of air drying

TREATMENT	MEAN	
	ACTUAL	TRANSFORMED
control	1.00	1.21[ab]
isolate 1	0.67	1.08[abc]
isolate 2	0.99	1.22[a]
isolate 3	0.32	0.91[c]
isolate 4	0.20	0.83[c]
isolate 5	0.38	0.93[bc]
isolate 6	0.37	0.92[c]
isolate 7	0.53	1.01[abc]
isolate 8	0.75	1.09[abc]
isolate 9	0.30	0.89[c]
isolate 10	0.25	0.82[c]

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

The study, aimed to determine the most beneficial bacteria and the standard dilution required forthe bacteria to work effectivelyin improving plant growth and rducingclubroot severity. The seedling experiment utilized the CRD Factorial while pot experiment followed CRD.

Seedling experiment revealed that six isolates from the dilution of 10^4 , six isolates from the dilution of 10^5 and three isolates from the dilution of 10^6 enhanced seed germination rate of chinese cabbage to 100%. Likewise, isolates nine and ten sigificantly improved the hieght of seeedlings using dilutions 10^4,10^5and10^6.

On the other hand, pot experiment showed that isolates 3, 4, 5, 6, 1 and 9 significantly increased the nitrogen content of the soil after harvest. In addition, isolates 10, 3 and 9 gave the lowest percent clubrootinfection of 26.66 %, 40 % and 46.67%. The same isolates gave the lowest club root severity infection of 2.3 and 2.67. In tems of vegetative fresh weight, isolate 1 gave the highest.

From the results, some bacterial isolates and actinomycetes-like organism from sunflower soil improved seed germination rate and plant height, enhanced nitrogen uptake and reduced club root severity.

Conclusion

Some isolates showed good effect on the growth and development of chinese cabbage and were able to reduced clubroot infection and severity. Isolates 2 and 8 enhanced the height of chinese cabbage. While isolates 1 and 8improved the vegetative weight of chinese cabbage. Percentage (%) nitrogen content analysis also showed that presence of isolate 1,2,3,4,5,6,9 in the soil enhanced the availability of nitrogen in the soil.Indicative that such isolates are good biofertilizers candidates. On the other hand Isolates 3, 9 and 10 reduced club root severity of chinese cabbage so they can be used to manage clubroot.

Recomendations

Based on the results, the following are the recommendations:

1. Characterizethe bacterial isolates that has a good effect on the growth of c. cabbage and isolates that lessened the club root severity.
2. Nitrogen analysis of sample plants must be conducted to support the result of nitrogen analysis from soils.
3. Conduct a study to find out if combination of two or more different isolate having different effect is applicable to attain better result.
4. Determine the link which creates antagonism of beneficial bacteria and*Plasmodiophorabrassicae*.
5. Conduct field experiment utilizing the treatments.

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APPENDICES

Appendix Table 1. Percentage germination as affected by the different isolates and the different dilution rates taken after two weeks

TREATMENTS	REPLICATES			TOTAL	MEAN
	R_1	R_2	R_3		
control	100%	100%	80%	280%	93.33%
isolate 1	60%	60%	100%	220%	73.33%
isolate 2	100%	60%	40%	200%	66.67%
isolate 3	40%	40%	40%	120%	40.00%
isolate 4	100%	80%	60%	240%	80.00%
isolate 5	80%	80%	80%	240%	80.00%
isolate 6	80%	80%	100%	260%	86.67%
isolate 7	60%	80%	80%	220%	73.33%
isolate 8	20%	100%	60%	180%	60.00%
isolate 9	40%	40%	60%	140%	46.67%
isolate 10	20%	40%	20%	80%	26.66%

Appendix Table 2.Seedling height (in) as affected by the different isolates and the different dilution rates measured after six weeks

TREATMENTS	REPLIICATIONS				TOTAL	MEAN
	R_1	R_2	R_3	R_4		
T_0D_0	2.53	2.23	2.69	2.58	10.03	2.51
T_1D_1	2.33	2.99	3.43	3.2	11.95	2.99
T_1D_2	4.3	3.7	3.73	4.3	16.03	4.01
T_1D_3	3.76	3.71	4.06	3.61	15.14	3.79
Sub-Total	10.39	10.4	11.22	11.11	43.12	10.79
T_2D_1	3.28	2.4	2.93	2.83	11.44	2.86
T_2D_2	3.1	3.18	3.16	3.49	12.93	3.23
T_2D_3	3	3.44	3.94	3.8	14.18	3.55
Sub-Total	9.38	9.02	10.03	10.12	38.55	9.64
T_3D_1	3.64	4.14	3.94	4.21	15.93	3.98
T_3D_2	4.19	4.04	4.46	4.35	17.04	4.26
T_3D_3	4.59	4.19	4.6	3.6	16.98	4.25
Sub-Total	12.42	12.37	13	12.16	49.95	12.49
T_4D_1	3.43	3.93	3.96	3.63	19.93	4.98
T_4D_2	4.08	3.8	3.76	3.29	14.93	3.73
T_4D_3	3.58	3.16	3.38	3.16	13.28	3.32
Sub-Total	11.09	10.89	11.1	10.08	48.14	12.03
T_5D_1	2.83	2.47	2.71	2.84	10.85	2.71
T_5D_2	3.04	3.3	3.25	3.51	13.1	3.28
T_5D_3	3.66	3.54	3.56	3.36	14.12	3.53
Sub-Total	9.53	9.31	9.52	9.71	38.07	9.52
T_6D_1	2.26	2.39	3.36	4.43	12.44	3.11
T_6D_2	4.33	3.88	3.97	3.86	16.04	4.01
T_6D_3	3.75	3.43	4.34	3.1	14.62	3.66
Sub-Total	10.34	9.7	11.67	11.39	43.1	10.78
T_7D_1	3.69	2.19	3.34	3.44	12.66	3.17
T_7D_2	3.44	3.55	3.96	3.91	14.86	3.72
T_7D_3	4.11	435	3.8	3.7	15.96	3.99
Sub-Total	11.24	440.74	11.1	11.05	43.48	10.88
T_8D_1	2.84	2.73	3.11	3.14	11.82	2.96
T_8D_2	3.77	3.97	3.2	3.18	14.12	3.53
T_8D_3	4.09	2.42	4.01	2.63	13.15	3.29
Sub-Total	10.7	9.12	10.32	8.95	39.09	9.78
T_9D_1	3.18	3.44	3.48	4.4	14.5	3.63
T_9D_2	3.96	4.66	4.66	4.41	17.69	4.42
T_9D_3	4.23	4.38	4.44	3.73	16.78	4.2
Sub-Total	11.37	12.48	12.58	12.54	48.97	12.25
$T_{10}D_1$	4.11	3.35	3.8	5.34	16.6	4.15
$T_{10}D_2$	4.76	5.26	5.39	4.61	20.02	5.01
$T_{10}D_3$	4.67	4.31	5.43	4.22	18.63	4.66
Sub-Total	13.54	12.92	14.62	14.17	55.25	13.82

SIMPLIFIED DATA

TREATMENTS	DILUTION RATE			MEAN
	10^4	10^5	10^6	
Control	N/A	N/A	N/A	2.51
Isolate 1	2.99	4.01	3.79	3.60[d]
Isolate 2	2.86	3.23	3.55	3.21[d]
Isolate 3	3.98	4.26	4.25	4.16[b]
Isolate 4	4.98	3.73	3.32	4.01[d]
Isolate 5	2.71	3.28	3.53	3.17[d]
Isolate 6	3.11	4.01	3.66	3.59[cd]
Isolate 7	3.17	3.72	3.99	3.63[d]
Isolate 8	2.96	3.53	3.29	3.26[d]
Isolate 9	3.63	4.42	4.20	4.08[bc]
Isolate 10	4.15	5.01	4.66	4.61[a]
MEAN	3.454[b]	3.92[a]	3.824[a]	

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

ANALYSIS OF VARIANCE

Source of variance	DF	SS	MS	Fc	Probability
Factor A	9	20.332	2.259	8.1971	0.0000
Factor B	2	9.808	4.904	17.7941	0.0000
AB	18	4.179	0.232	0.8424	
Error	90	24.803	0.276		
Total	119	59.122			

Coefficient of Variation- 14.09 %

Appendix Table 3. Root length (in) as affected by the different isolates and the different dilution rates measured after six weeks

Treatment/Dilution	REPLICATIONS				Total	Mean
	R_1	R_2	R_3	R_4		
T_0D_0	5.53	07.23	07.63	10.07	30.46	7.62
T_1D_1	7.43	09.77	10.05	12.40	39.65	9.91
T_1D_2	7.90	12.3	9.03	11.67	40.9	10.23
T_1D_3	8.03	09.9	9.70	06.10	33.73	8.43
Sub-Total	23.36	31.97	28.78	30.17	114.28	28.57
T_2D_1	10.43	9.27	12.7	8.2	40.6	10.15
T_2D_2	10.70	10.9	9.3	8.03	38.93	9.73
T_2D_3	11.77	9.97	13.7	8.1	43.54	10.89
Sub-Total	32.9	30.14	35.7	24.33	123.07	30.77
T_3D_1	8.30	8.03	9.17	7.6	15.9	8.28
T_3D_2	9.70	9.87	8.7	10	19.7	9.57
T_3D_3	11.67	9.3	8.33	9.9	21.57	9.8
Sub-Total	29.67	27.2	26.2	27.5	57.17	27.65
T_4D_1	7.5	13.6	12.17	9.83	43.1	10.78
T_4D_2	11.2	10.7	11.6	10.73	44.23	11.06
T_4D_3	9.93	12.6	8.37	13.03	43.93	10.98
Sub-Total	28.63	36.9	32.14	33.59	131.26	32.82
T_5D_1	11.13	11.87	10.53	10.63	44.16	11.04
T_5D_2	11.8	13.13	8.23	8.6	41.76	10.44
T_5D_3	10.63	13.57	11.87	10.3	46.37	11.59
Sub-Total	33.56	38.57	30.63	29.53	132.29	33.07
T_6D_1	9.77	11.07	11.33	10.53	42.7	10.68
T_6D_2	11.33	9.23	13.53	12.47	46.56	11.64
T_6D_3	10.63	15.47	10.77	10.33	47.2	11.8
Sub-Total	31.73	35.77	35.63	33.33	136.46	34.12
T_7D_1	8.77	8.57	11.4	10.1	38.84	9.71
T_7D_2	11.4	5.93	6.93	8.73	32.99	8.25
T_7D_3	11.63	14.53	7.77	8.83	42.76	10.69
Sub-Total	31.8	29.03	26.1	27.66	114.59	28.65
T_8D_1	10.37	8.3	14.57	10.2	43.44	10.86
T_8D_2	9.16	16.37	10.47	9.9	45.9	11.48
T_8D_3	7.54	9.23	10.63	13.53	40.93	10.23
Sub-Total	27.07	33.9	35.67	33.63	130.27	32.57
T_9D_1	11.63	8.3	12.93	10.27	43.13	10.78
T_9D_2	9.47	8.33	11.67	8.7	38.17	9.54
T_9D_3	7.57	8.43	8.03	17.8	41.83	10.46
Sub-Total	28.67	25.06	32.63	36.77	123.13	32.57
$T_{10}D_1$	10.23	10.83	11	12.1	44.16	11.04
$T_{10}D_2$	11.27	11.73	8.4	14.1	45.5	11.38
$T_{10}D_3$	11.67	10.33	13.6	9.87	45.47	11.37
Sub-Total	33.17	32.89	33	36.07	135.13	33.79

SIMPLIFIED DATA

TREATMENTS	DILUTION RATE			MEAN
	10^4	10^5	10^6	
Control	N/A	N/A	N/A	07.62
Isolate 1	09.91	10.23	08.43	09.52
Isolate 2	10.15	09.73	10.89	10.26
Isolate 3	08.28	09.57	09.80	09.22
Isolate 4	10.78	11.06	10.98	10.94
Isolate 5	11.04	10.44	11.59	11.02
Isolate 6	10.68	11.64	11.80	11.37
Isolate 7	09.71	08.25	10.69	09.55
Isolate 8	10.86	11.48	10.23	10.86
Isolate 9	10.78	11.38	10.46	10.87
Isolate 10	11.04	09.54	11.37	10.65
MEAN	10.323	10.332	10.624	

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	Fc	Tabulated F	
					.05	.01
Factor A	9	124.983	13.887	0.9269	1.98	2.61
Factor B	2	14.110	7.055	0.4709		
AB	18	192.109	10.673	0.7124		
Error	90	1348.413	14.982			
Total	119	1679.615				

Coefficient of Variation- 35.79 %

Appendix Table 4. Initial and final percentage nitrogen content of soil samples, taken before the introduction of bacteria and after harvest

TREATMENTS	REPLICATIONS			TOTAL	MEAN
	R_1	R_2	R_3		
Initial samples	0.23	0.25	0.22	0.70	0.23^{c}
T_0 - control	0.25	0.24	0.23	0.72	0.24^{bc}
T_1 – isolate 1	0.30	0.32	0.31	0.93	0.31^{a}
T_2 - isolate 2	0.30	0.31	0.30	0.91	0.30^{a}
T_3 – isolate 3	0.29	0.37	0.35	1.01	0.34^{a*}
T_4 – isolate 4	0.30	0.39	0.29	0.98	0.33^{a}
T_5 – isolate 5	0.30	0.37	0.31	0.98	0.33^{a}
T_6 – isolate 6	0.32	0.35	0.26	0.93	0.31^{a}
T_7 – isolate 7	0.31	0.32	0.24	0.87	0.29^{ab}
T_8 – isolate 8	0.30	0.31	0.23	0.84	0.28^{abc}
T_9 – isolate 9	0.29	0.30	0.32	0.91	0.30^{a}
T_{10} – isolate 10	0.27	0.33	0.26	0.86	0.29^{ab}
Grand Total				10.640	
Grand mean					0.296

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	Fc	Tabulated F	
					.05	.01
Treatments	11	0.034	0.003	3.000*	2.22	3.09
Error	24	0.028	0.001			
Total	35	0.063				

Coefficient of variation = 8.95

*= Significant at 5% level of significance

Appendix Table 5. Effect of the different Isolates on the final height (inches) of Chinese cabbage taken after three months

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	6.5	6.4	8.0	20.90	6.97
T_1 – isolate 1	7.1	8.5	8.0	23.60	7.87
T_2 - isolate 2	8.5	8.6	7.5	24.60	8.20
T_3 – isolate 3	6.8	6.6	7.5	20.90	6.97
T_4 – isolate 4	0.0	6.5	7.5	14.0	4.67
T_5 – isolate 5	6.5	6.6	8.3	21.40	7.13
T_6 – isolate 6	8.9	8.5	0.0	17.40	5.80
T_7 – isolate 7	7.6	6.7	8.6	22.90	7.63
T_8 – isolate 8	6.0	11	9.0	26.00	8.67
T_9 – isolate 9	7.0	6.5	8.7	22.20	7.40
T_{10} – isolate 10	6.5	7.9	7.0	21.40	7.13

TRANSFORMED DATA

TREATMENTS	RIPLICATIONS			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	2.65	2.62	2.92	8.19	2.73
T_1 – isolate 1	2.75	3.00	2.92	8.67	2.89
T_2 - isolate 2	3	3.02	2.83	8.85	2.95
T_3 – isolate 3	2.70	2.66	2.83	8.19	2.73
T_4 – isolate 4	0.71	2.65	2.83	6.19	2.06
T_5 – isolate 5	2.65	2.66	2.97	8.28	2.76
T_6 – isolate 6	3.07	3.00	0.71	6.78	2.26
T_7 – isolate 7	2.85	2.68	3.02	8.55	2.85
T_8 – isolate 8	2.55	3.39	3.08	9.02	3.01
T_9 – isolate 9	2.73	2.65	3.03	8.41	2.80
T_{10} – isolate 10	2.65	2.90	2.74	8.29	2.76

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	2.499	0.2499	0.78	0.6510
TRT	10	2.499	0.2499	0.78	0.6510
Error	32	7.092	0.32223		
Corrected Total	32	9.591			

Coefficient of Variation = 20.95280%

Appendix Table 6. Percentage incidence of club root as affected by the different isolates taken at harvest

TREATMENTS	REPLICATIONS			Total	mean
	R_1	R_2	R_3		
T_0 - control	100%	100%	80%	280%	93.33%
T_1 – isolate 1	60%	60%	100%	220%	73.33%
T_2 - isolate 2	100%	60%	40%	200%	66.67%
T_3 – isolate 3	40%	40%	40%	120%	40%
T_4 – isolate 4	100%	80%	60%	240%	80%
T_5 – isolate 5	80%	80%	80%	240%	80%
T_6 – isolate 6	80%	80%	100%	260%	86.67%
T_7 – isolate 7	60%	80%	80%	220%	73.33%
T_8 – isolate 8	20%	100%	60%	180%	60%
T_9 – isolate 9	40%	40%	60%	140%	46.67%
T_{10} – isolate 10	20%	40%	20%	80%	26.66%

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	12921.21212	1292.12121	3.44	0.0075
TRT	10	12921.21212	1292.12121	3.44	0.0075
Error	32	8266.66667	375.75758		
Corrected Total	32	21187.87879			

Coefficient of Variation = 29.34346%

Appendix Table 7. Club root severity as affected by the different isolates, taken at harvest

TREATMENTS	REPLICATIONS			Total	Mean
	R_1	R_2	R_3		
T_0 - control	6	5	5	16	5.33^{a}
T_1 – isolate 1	3	4	5	12	4^{abc}
T_2 - isolate 2	5	4	2	11	3.67^{abc}
T_3 – isolate 3	3	3	2	8	2.67^{c}
T_4 – isolate 4	6	4	4	14	4.67^{ab}
T_5 – isolate 5	5	5	4	14	4.67^{ab}
T_6 – isolate 6	4	4	6	14	4.67^{ab}
T_7 – isolate 7	3	4	4	11	3.67^{abc}
T_8 – isolate 8	2	4	3	9	3^{bc}
T_9 – isolate 9	3	3	2	8	2.67^{c}
T_{10} – isolate 10	2	3	2	7	2.33^{c}

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	30.061	3.0061	3.67	0.0052
TRT	10	30.061	3.0061	3.67	0.0052
Error	32	18.00	0.8182		
Corrected Total	32	48.061			

Coefficient of Variation = 24.07228%

Appendix Table 8. Fresh weight of vegetative parts (g) taken at harvest

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	50	13	19	82.0	27.33
T_1 – isolate 1	82	123	95	300	100
T_2 - isolate 2	35	88	60	183	61.00
T_3 – isolate 3	50	36	87	173	57.67
T_4 – isolate 4	0.0	21	30	51.0	17.00
T_5 – isolate 5	31	19	48	98.0	32.67
T_6 – isolate 6	81	69	0.0	150	50.00
T_7 – isolate 7	59	45	57	161	53.67
T_8 – isolate 8	30	135	56	221	73.67
T_9 – isolate 9	53	16	83	152	50.67
T_{10} – isolate 10	40	47	47	134	44.67

TRANSFORMED DATA

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	7.11	3.67	4.42	15.2	5.07
T_1 – isolate 1	9.08	11.11	9.77	29.96	9.99
T_2 - isolate 2	5.96	9.41	7.78	23.15	7.72
T_3 – isolate 3	7.11	6.04	9.35	22.5	7.5
T_4 – isolate 4	0.71	4.64	5.52	10.87	3.62
T_5 – isolate 5	5.61	4.42	6.96	16.99	5.66
T_6 – isolate 6	9.03	8.34	0.71	18.08	6.03
T_7 – isolate 7	7.71	6.75	7.58	22.04	7.35
T_8 – isolate 8	5.52	11.64	7.52	24.68	8.23
T_9 – isolate 9	7.31	4.06	9.14	20.51	6.84
T_{10} – isolate 10	6.36	6.89	6.89	20.14	6.71

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	86.4394	8.6439	3.69	0.1474
TRT	10	86.4394	8.6439	3.69	0.1474
Error	32	122.7707	5.1259		
Corrected Total	32	199.2100			

Coefficient of Variation = 33.33650%

Appendix Table 9. Dry weight of vegetative parts (g), obtained seven weeks after air drying

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	3.41	3.06	1.89	8.36	2.79
T_1 – isolate 1	5.01	4.72	5.91	15.64	5.21
T_2 - isolate 2	2.18	4.37	2.24	8.79	2.93
T_3 – isolate 3	2.70	1.26	3.89	7.85	2.62
T_4 – isolate 4	0.00	0.90	1.36	2.26	0.75
T_5 – isolate 5	1.30	1.16	2.11	4.57	1.52
T_6 – isolate 6	3.51	2.44	0.00	5.95	1.98
T_7 – isolate 7	3.17	3.08	3.37	9.62	3.21
T_8 – isolate 8	2.01	4.68	2.78	9.47	3.16
T_9 – isolate 9	2.34	0.96	3.06	6.36	2.12
T_{10} – isolate 10	1.99	0.58	2.13	4.70	1.57

TRANSFORMED DATA

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	1.98	1.89	1.55	5.42	1.81
T_1 – isolate 1	2.35	2.28	2.53	7.16	2.38
T_2 - isolate 2	1.64	2.21	1.66	5.51	1.84
T_3 – isolate 3	1.79	1.33	2.10	5.22	1.74
T_4 – isolate 4	0.71	1.18	1.86	3.75	1.25
T_5 – isolate 5	1.34	1.29	1.62	4.25	1.42
T_6 – isolate 6	2.00	1.72	0.71	4.43	1.48
T_7 – isolate 7	1.92	1.89	1.97	5.78	1.93
T_8 – isolate 8	1.58	2.28	1.81	5.67	1.89
T_9 – isolate 9	1.69	1.21	1.89	4.79	1.60
T_{10} – isolate 10	1.58	1.04	1.62	4.24	1.41

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	3.0480	0.3048	2.23	0.0564
TRT	10	3.0480	0.3048	2.23	0.0564
Error	32	3.0103	0.1368		
Corrected Total	32	6.0583			

Coefficient of Variation = 21.71296%

Appendix Table 10. Fresh weight of roots (g) taken at harvest

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	4.82	0.64	2.86	8.32	2.77
T_1 – isolate 1	1.01	0.96	1.92	3.89	1.30
T_2 - isolate 2	2.25	1.98	3.66	7.89	2.63
T_3 – isolate 3	0.40	0.58	1.01	1.99	0.66
T_4 – isolate 4	0	0.57	1.97	2.54	0.85
T_5 – isolate 5	3.15	0.25	0.89	4.29	1.43
T_6 – isolate 6	1.64	0.59	0	2.23	0.74
T_7 – isolate 7	0.62	0.59	2.01	3.22	1.07
T_8 – isolate 8	0.43	4.07	1	5.5	1.83
T_9 – isolate 9	0.48	0.35	1.25	2.08	0.69
T_{10} – isolate 10	1.12	0.67	0.45	2.24	0.75

TRANSFORMED DATA

TREATMENTS	REPLICATION			Total	mean
	R_1	R_2	R_3		
T_0 - control	2.31	1.07	1.83	5.21	1.74
T_1 – isolate 1	1.23	1.21	1.56	4	1.33
T_2 - isolate 2	1.66	1.57	2.04	5.27	1.76
T_3 – isolate 3	0.95	1.04	1.23	3.22	1.07
T_4 – isolate 4	0.71	1.03	1.57	3.31	1.10
T_5 – isolate 5	1.91	0.87	1.18	3.96	1.32
T_6 – isolate 6	1.46	1.04	0.71	3.21	1.07
T_7 – isolate 7	1.06	1.04	1.58	3.68	1.23
T_8 – isolate 8	0.96	2.14	1.22	4.32	1.44
T_9 – isolate 9	0.99	0.92	1.32	3.23	1.08
T_{10} – isolate 10	1.27	1.17	0.98	3.42	1.14

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	1.9323	0.19323	1.27	0.3048
TRT	10	1.9323	0.19323	1.27	0.3048
Error	32	3.3461	0.1521		
Corrected Total	32	5.2784			

Coefficient of Variation = 30.04843%

Appendix Table 11.Dry weight of Roots (g) recorded after seven weeks of air drying

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	0.98	0.43	1.58	3	1
T_1 – isolate 1	0.74	0.45	0.82	2.01	0.67
T_2 - isolate 2	0.97	0.90	1.09	2.96	0.99
T_3 – isolate 3	0.28	0.36	0.33	0.97	0.32
T_4 – isolate 4	0.00	0.31	0.29	0.6	0.2
T_5 – isolate 5	0.46	0.12	0.55	1.13	0.38
T_6 – isolate 6	0.59	0.51	0	1.1	0.37
T_7 – isolate 7	0.32	0.42	0.84	1.58	0.53
T_8 – isolate 8	0.35	1.46	0.43	2.24	0.75
T_9 – isolate 9	0.11	0.24	0.55	0.9	0.3
T_{10} – isolate 10	0.19	0.32	0.24	0.75	0.25

TRANSFORMED DATA

TREATMENTS	REPLICATION			TOTAL	MEAN
	R_1	R_2	R_3		
T_0 - control	1.22	0.96	1.44	3.62	1.21
T_1 – isolate 1	1.11	0.97	1.15	3.23	1.08
T_2 - isolate 2	1.21	1.18	1.26	3.65	1.22
T_3 – isolate 3	0.88	0.93	0.91	2.72	0.91
T_4 – isolate 4	0.71	0.9	0.89	2.5	0.83
T_5 – isolate 5	0.98	0.79	1.02	2.79	0.93
T_6 – isolate 6	1.04	1	0.71	2.75	0.92
T_7 – isolate 7	0.91	0.96	1.16	3.03	1.01
T_8 – isolate 8	0.92	1.4	0.96	3.28	1.09
T_9 – isolate 9	0.78	0.86	1.02	2.66	0.89
T_{10} – isolate 10	0.69	0.91	0.86	2.46	0.82

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	DF	SS	MS	F value	Pr> F
MODEL	10	0.5909	0.05909	2.66	0.0269
TRT	10	0.5909	0.05909	2.66	0.0269
Error	32	0.4891	0.02223		
Corrected Total	32	1.07999			

Coefficient of Variation = 15.05225%