MANAGEMENT OF CLUBROOT (*Plasmodiophora brassicae Wor.*) on CABBAGE USING *Trichoderma* KA AND LIME IN NATUBLENG, BUGUIAS, BENGUET

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ABSTRACT

The study validated the effect of *Trichoderma* KA as biological control against clubroot on cabbage (Scorpio variety) in seedbed and under field condition. Rates of 10,15 and 30 g and 10, 15 and 20 g of *Trichoderma* at spore concentrations of 1.4 x 10_6 were applied in 1 x 10 m_2 in seedbed and in field plots, respectively. Lime was applied at 6 t ha_{-1} in plots where it is needed while flusulfamide at 300 kg/ha served as the check fungicide.Seeds were sown and seedlings were transplanted two weeks after the application of *Trichoderma* KA. Clubroot severity, incidence, percent clubroot control, fresh top and root weight, oven dry top and root weight and yield were recorded.

In the seedbed, 30 g *Trichoderma* effected the lowest clubroot severity and incidence and provided a control of 89.50% and was comparable to flusulfamide. Eighty-two days after transplanting in the field, the treatment comparable to the check was 20 g *Trichoderma* KA plus CaO. Plants receiving this treatment had the highest heaviest roots and tops. At harvest, this treatment also effected the widest polar circumference of 47.91 cm and produced the highest mean marketable yield of 9.5% t ha_{-1} with 80.8% clubroot control. This treatment is comparable to flusulfamide.

INTRODUCTION

Clubroot (*Plasmodiophora brassicae*) is an obligate parasite and one of the most damaging disease of cabbage and difficult to control. Affected plants whose main symptoms include yellowing, wilting, and stunting are unable to produce quality heads (Agrios, 1978). Infection starts with the germination of cyst in response to allyl isothiocyanate from crucifer exudates. Primary zoospores are released that infect host root hairs. In the roots, a multinucleate plasmodium develops, divides, and forms the secondary zoospores that later on infect nearby healthy plants. The pathogen survives in the soil as dormant cyst for up to eight years without the host (Karling 1998). Cool, wet, and acidic soils provide the most favorable environment for the pathogen. In acidic soils, the temperature range for infection is between 50 and 95 $_o$F (10 and 35 $_o$C) and lower in alkaline soils. As the disease progresses, it may lead to plant death creating an inoculum that may persist in the soil for a long time (Tremblay *et al.*, 2005).

In Benguet, Mariano *et al*., (1990) reported the presence of the disease in 18 cabbage- growing areas distributed in Atok, Buguias, Baguio, La Trinidad and Kibungan. Similarly, Nagpala *et al.,* (2004) noted in their survey the presence of clubroot in the 6.11 ha planted with cabbage in Atok and Buguias with a severity infection ranging from 30 to 100%.This is attributed to the crop production practices of farmers such as mono cropping, incorrect nitrogen fertilizer application which influence soil acidity and improper sanitation whereby infected roots are left in the field after harvest.

The common management of clubroot practiced by cabbage growers is the application of lime to raise the soil pH to neutral based on the fact that spores does not germinate on alkaline condition and the use of protectant soil fungicide in order to inhibit spore germination of the pathogen. However, some farmers are slowly shifting to good agriculture practice and adopted the use of biological control agents, one of which is the use of *Trichoderma*. Aside from its decomposing ability, it acts as mycoparasite against plant pathogenic fungi such as *Fusarium* (Nagpala, 1999), *Sclerotium*, *Pyhtium*, *Rhizoctonia*, and as solubilizer of macro and micronutrients to improve plant growth (Singh, 2010).

Thus, the study aims to evaluate the local isolate *Trichoderma* KA and to determine its clubroot control and its effect on the growth and yield of cabbage, Scorpio variety.

METHODOLOGY

Experiment I: Seedbed Experiment

Seedbed Preparation. An area of about 50 m_2 naturally and highly infected with clubroot was prepared for the study. It was divided into five plots measuring 1 x 5 m_2. Before sowing cabbage seeds of the variety Scorpio, the different treatments were laid out using the complete randomized design (CRD) with three replications.

Treatments and Field Application. The *Trichoderma* strain, referred to as *Trichoderma* KA, at a spore concentration of 1.4 x 106 spores ml_{-1} was used biological control agent in the study. The treatments were as follows:

	Treatment Rate Formulation		Application	
T_0	CaO (Control)	6 t ha_{-1}	Dust	15 days before sowing
T_1	*Trichoderma* KA	10 ml Spore	suspension	15 days before sowing
T_2	*Trichoderma* KA + CaO	20 ml	Spore suspension	15 days before sowing
T_3	*Trichoderma* KA+ CaO	30 ml +6 t ha_{-1}	Spore suspension+dust	15 days before sowing
T_4	Flusulfamide	300 kg ha_{-1}	Dust	One day before sowing

Experiment II. Field Experiment

Land Preparation. An area of about 200 m_2 naturally and highly infected with *Plasmodiophora brassicae* (clubroot) was thoroughly prepared for the study. Unprocessed chicken manure was incorporated into the soil at a rate of 8 t ha_{-1}. The area was divided into four blocks with 8 plots each measuring 1 x 10 m_2. The different treatments were laid out using the Randomized Complete Block Design with four replications. Lime (6 t ha_{-1}) was applied one month before transplanting

Treatments and Field application. The biocontrol agent was applied in plots two weeks before transplanting.

	Treatments	Rate	Application
T_0	14-14-14+urea + CaO (farmers practice)	800kg + 6 t ha_{-1}	Two weeks after transplanting and at hilling up
T_1	*Trichoderma* KA	10 ml	15 days before planting
T_2	*Trichoderma* KA	10 ml + 6 t ha_{-1}	15 days before planting
T_3	*Trichoderma* KA	15 ml	
T_4	*Trichoderma* KA+ CaO	15 ml + 6 t ha_{-1}	15 days before planting
T_5	*Trichoderma* KA	20 ml	
T_6	*Trichoderma* +CaO	20 ml + 6 t ha_{-1}	15 days before planting
T_7	Flusulfamide	300 kg ha_{-1}	One day before planting
T_8	CaO	6 t ha_{-1}	One month before planting

Clubroot Severity Rating Scale.With slight modification, the rating of clubroot severity followed the scale of Williams (1966). Photos illustrate the size of clubroot (Figure 1):

Scale	Description
D.	Normal growth (no clubbing)
E.	Slight clubbing on lateral roots
F.	Moderate clubbing on lateral roots
(5)	Major clubbing on lateral and tap roots

1 2

3 4

Figure 1. Visual rating of clubroot infection on cabbage seedlings

Planting. Healthy cabbage seedlings from the treated plot with Trichoderma were transplanted in a double row method with a distance of 30 cm between rows and 30 cm between hills. The seedlings were transplanted to these plots accordingly following the treatment assignment of the seedbed where they were obtained. The initial pH of the soil in the area was 5.2.

Data Gathered

7) Disease severity. Sample plants per replicate were selected at random and then uprooted. The severity of clubroot was determined using the rating scale and the rating was recorded.

Disease severity in the seedbed (45 DAE).

Fifty sample plants per replicate were choosen at random 45 days after emergence.

b. Disease severity in the field (82 DAT). Ten (sample plants per replicate were choosen at random and uprooted 82 days after transplanting.

1. Clubroot incidence (% infection).Using the same sample plants uprooted from the seedbed and, the number of infected plants was counted and then used to compute % infection as follows:

$$\text{infection} = \frac{\text{Total no. of plants/plot} - \text{no. of infected plants} \times 100}{\text{Total no. of plants}}$$

Percent disease control. This was computed by using the formula:

4. $$\text{disease control} = \frac{\text{Severity rating of untreated - severity rating of treated}}{\text{Severity rating of untreated}} \times 100$$

4. Fresh and dry Root weight (g). Fresh cabbage roots were separated from cabbage heads and were weighed.

5. Fresh and oven dry weight of cabbage heads (g). This was done by harvesting cabbage heads from 10 randomly selected plants per plot then taking the fresh and oven dry weight after two weeks of drying in an oven at a temperature of 70 ^{o}C for 24 hours.

6. Head circumference (cm). At harvest, ten plants from each plot were selected at random and the polar and equatorial circumferences were measured and recorded.

7. Yield (t ha^{-1}).The marketable and non-marketable cabbage heads at harvest were weighed per treatment

RESULTS AND

DISCUSSION Seedbed Experiment

Clubroot Severity. There were significant differences noted on the clubroot severity rating of cabbage seedlings applied with *Trichoderma* at different rates to that of the farmers' practice (Table 1). Plants with the highest clubroot severity infection of 3.10 % received lime only. On the other hand, seedlings treated with the fungicide (flusulfamide) at 300 kg ha^{-1} and 30 g *Trichoderma* combined with 6 t ha^{-1} lime had the lowest clubroot severity of 1%.

The result showed that applying 10 g of *Trichodem*a significantly reduce clubroot severity compared to lime alone. Chet *et al*. (2006) stated that *Trichoderma* spp. colonizes and penetrates plant root tissues and initiate a series of morphological and biochemical changes in the plant. This change is later considered as plant defense response, which then leads to induced systemic resistance (ISR) in the entire plant.

Trichoderma grows on the surface of roots where it provides protection and enhances root growth. Its spores survive in the soil, living on food mostly secreted from the root surface. Since the fungus multiplies on its own, a small amount will grow to continually cover the roots, thus, protecting all the roots for the entire growing season (Bjorkman, 1999).

Clubroot Infection

There are no clubbing observed in plants applied with 30 g *Trichodrem*a + 6 t ha^{-1} lime and with the flusulfamide at 300 kg ha^{-1} (Table 1). On the other hand, the highest clubroot infection of 30% was recorded on plants applied with lime.

Even at the lowest rate 10 g, *Trichoderma* is more effective in suppressing clubroot infection than lime application alone. Further, as the rate of *Trichoderma* application increased the infection decreases resulting in significant differences among treatments.

Clubroot Control in Seedbed

All the treatments affected significantly better disease control than CaO alone (Table 1).

The combination of 30 g *Trichoderma* with lime is effective in suppressing the growth of clubroot in seedbed similar to the fungicide (flusufamide).The *Trichoderma* may have reduced the clubroot inoculum in the soil, thus resulting to lesser infection, which ultimately expresses to greater control. Aziz (1996) observed that application of *Trichoderma* spp. in the form of wheat-bran preparation, conidial suspension or seed coating reduced the pathogen counts in the rhizosphere soil of bean.

Field Experiment

Clubroot Severity. There are no significant differences in clubroot severity among the plants treated with *Trichoderma* and with flusulfamide. The clubbing in these treatments in turn was significantly lower from the farmers practice. These results indicate that lowest rate (10 g) can be also used to control clubroot in the field.

The application of *Trichoderma* at different rates from 10 to 30 gin field plots augmented what's already present on the root surface of seedlings applied in the seedbed. Further, Kubicek and Harman (1998) observed that *Trichoderma* treated plants have the ability to solubilize and sequester inorganic nutrients thereby inducing resistance to plants against diseases. Similarly, Cadap (2001) noted that soils applied with *Trichoderma* KA increased the initial pH from 5.82 to 6.17. Spores of *Plasmodiophora brassicae* is prevented from germination when soil pH is raised close to neutral. The possibility of induced resistance in cabbage inoculated with *Trichoderma* regardless of the amount applied and increased soil pH are viewed as

Table 1. Mean percent severity, incidence and clubroot control on cabbage seedlings after 60 days as affected by the application of *Trichoderma* KA

TREATMENTS	CLUBROOT SEVERITY	CLUBROOT INCIDENCE (%)	CLUBROOT CONTROL (%)
Lime alone (CaO) (6 t ha^{-1})	3.10^{a}	30.00^{a}	-
10 ml *Trichoderma*	1.86^{b}	10.67^{b}	80.33^{c}
20 ml *Trichoderma*	1.53^{c}	6.67^{b}	83.83^{b}
30 ml *Trichoderma* + Lime (6 t ha^{-1})	1.00^{d}	0.00^{c}	89.50^{a}
Flusulfamide (300 kg ha^{-1})	1.00^{d}	0.00^{c}	89.50^{a}

Means with the same letter in a column are not significantly different at 5% level by DMRT

Table 2. Mean percent (%) disease severity of cabbage as affected by the application of *Trichoderma* KA

TREATMENTS	DISEASE SEVERITY	CLUBROOT CONTROL(%)
10 g *Trichoderma*	3.10^{b}	78.5^{abcd}
10 g *Trichoderma* + Lime (CaO) (6 t ha^{-1})	3.12^{b}	78.3^{abcd}
15 g *Trichoderma*	3.07^{b}	78.7^{abcd}
15 g *Trichoderma* +Lime (CaO) (6 t ha^{-1})	3.00^{b}	79.2^{abc}
20 g *Trichoderma*	2.95^{b}	79.5^{abc}
20 g *Trichoderma* + Lime (CaO) (6 t ha^{-1})	2.775^{b}	80.8^{ab}
Fungicide (Flusulfamide) (300 kg ha^{-1})	2.25^{bc}	82.6^{a}
Lime (CaO) alone (6 t ha^{-1})	3.10^{b}	78.5^{abcd}
Triple 14 + Urea and CaO (Farmers Practice)	3.60^{a}	-

Means with the same letter in a column are not significantly different at 5% level by DMRT.

contributory factors to low clubroot severity infection under field condition.

Percent Clubroot Control in the Field Plots

The effect of the different rates of *Trichoderma* against clubroot infection is shown in Table 5. The highest percent disease control of 82.60 % was obtained from the treatment applied with the fungicide (flusulfamide). These were followed by 80.8 % of the treatment applied with 20 g *Trichoderma* added with 6 t ha_{-1} lime (CaO). The treatments applied with 20 g *Trichoderma* alone and 15 g *Trichoderma* with lime provided clubroot control of 79.50 % and 79. 20 % respectively.

Those treatments with 10g *Trichoderma* only, 10 g *Trichoderma* added with lime and 15 g *Trichoderma* only had lower percentage control of 78.50 %, 78.30 % and 78.70 %, respectively.

There was also a reduction of clubroot incidence in the control (plants applied with triple 14 and urea) of about 78.50 % and that this result can be attributed to the late application of the nitrogenous fertilizers which were applied in the soil during heading stage. Such practice allowed the cabbage to form heads while delaying the onset of clubroot infection.

It is often observe that early application of nitrogenous fertilizer enhance the severity of clubroot infection under field condition. This result also proves that while the fungicide flusulfamide provided the highest clubroot control, the application of 20 g *Trichoderma* especially when added with lime can be as effective as the fungicide in protecting the cabbage roots against clubroot infection.

Statistical analysis showed that all the treatments with *Trichoderma* from 10 to 20 g with or without lime are effective in controlling clubroot in the field.

Root Weight (g)

Roots of plants applied with 15 g and 20 g *Trichoderma* plus lime had the highest fresh and dry weights (Table 3).These treatments significantly differed from the other treatments indicating further that the highest rate of *Trichoderma* with lime is more effective in promoting root growth than the treatment applied with fungicide.

The root weights from the treatments applied with the lower rate of *Trichoderma* with or without lime were similar with the farmers practice. Plant roots in these treatments had several infested lateral roots.

Unlike in the treatment with 20 g *Trichoderma* combined with lime, the main root remained vigorous indicating that lower rates of *Trichoderma* are less effective in promoting root growth.

Table 3. Fresh and oven dry root weight of cabbage (g) 82 days after transplanting as affected by the application of *Trcihoderma* KA

TREATMENTS	WEIGHT OF ROOTS	
	FRESH WEIGHT	OVEN DRY WEIGHT
10 g *Trichoderma*	93.75^{bc}	8.20^{b}
10 g *Trichoderma* + CaO (6 t ha^{-1})	106.25^{b}	8.70^{b}
15 g *Trichoderma*	112.50^{b}	8.00^{b}
15 g *Trichoderma* + CaO (6 t ha^{-1})	125.0^{a}	10.90^{a}
20 g *Trichoderma.*	106.25^{b}	10.10^{a}
20 g *Trichoderma* + CaO (6 t ha^{-1})	131.25^{a}	10.95^{a}
Fungicide (Flusulfamide) 300 kg ha^{-1}	87.50^{bc}	7.80^{b}
Lime (CaO) alone (6 t ha^{-1})	87.50^{b}	7.35^{b}
Triple 14 + Urea and CaO (Farmers practice)	100.00^{b}	6.10^{b}

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

According to Harman and Kubicek (1998), the fungus *Trichoderma* has the ability to increase the rate of plant growth and the production of more robust roots as a cosmopolitan fungi.

Trichoderma dominates in soil and this may be attributed to its diverse metabolic capability and aggressive competitiveness in nature (Lewis and Papavizas, 1991), Harman *et al*., (1996). These characteristicsmakethemgoodbiologicalcontrolagents.

Top Weight of Cabbage (kg)

The rate of application of *Trichoderma* influenced the fresh and oven dry top weight of the cabbage, (Table 4). The highest weight was obtained from the treatment applied with 20 g *Trichoderma* combined with 6 t ha $^{-1}$ lime.

The result was significantly different with the lower rates of *Trichoderma* (10 and 15 g) also added with lime. Those without lime were comparable with the farmers practice and lime alone.

The result implies that *Trichoderma* combined with lime has potential effect in controlling clubroot infection. The fresh weight indicates that the roots of the plants were functional in absorbing water from the soil and that the stem of the plants is also functional as transport avenue of water to the leaves.

Thus, the higher the fresh weight the healthier the plant as seen in the treatment with *Trichoderma* and lime.

Lime when applied in the soil is known to improve its biological properties. It also enhances the availability of soil nutrients for plant uptake.

Similarly, earlier studies showed that *Trichoderma* improved root growth due to enhanced nutrient uptake of mineral nutrients and increased soil pH. It is apparent that the performance of *Trichoderma* as growth promoter is enhanced with the addition of lime.

Head Circumference (cm) at Harvest

The different rates of *Trichoderma* application significantly affected the polar circumference of the cabbage but not on the equatorial circumference (Table 5).

The similarity of the polar circumference in all the treatment may indicate that the attribute is not affected by the treatments.

Plants applied with 15 and 20 g *Trichoderma* plus lime had the widest polar circumference of 47.91 and 47.39 cm, respectively. These were comparable to those plants applied with the flusulfamide but significantly wider than all the other treatments.

Table 4. Fresh top and oven dry weight of cabbage (kg) recorded 82 days after planting as affected by the application of *Trichoderma* KA

TREATMENTS	FRESH WEIGHT	OVEN DRY WEIGHT
10 g *Trichoderma*	0.520^{c}	0.085^{d}
10 g *Trichoderma* + CaO (6 t ha^{-1})	0.660^{ab}	0.160b^{c}
15 g *Trichoderma*	0.530^{c}	0.112^{bc}
15 g*Trichoderma* + CaO (6 t ha^{-1})	0.690^{ab}	0.192^{a}
20 g *Trichoderma*	0.570^{c}	0.127^{b}
20 g *Trichoderma* + CaO (6 t ha^{-1})	0.730^{a}	0.202^{a}
Fungicide (Flusulfamide) (300 kg ha^{-1})	0.640^{ab}	0.137^{b}
Lime (CaO) alone (6 t ha^{-1})	0.460^{cde}	0.081^{d}
Triple14 + Urea and CaO (Farmers Practice)	0.490^{cd}	0.084^{d}

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

Table 5. Mean polar and equatorial head circumference (cm) as affected by the application of *Trichoderma* KA

TREATMENTS	MEAN POLAR CIRCUMFERENCE (POLAR)	MEAN EQUATORIAL CIRCUMFERENCE (cm)
10 g *Trichoderma*	44.57^{b}	43.04^{abcd}
10 g *Trichoderma* + CaO (6 t ha^{-1})	44.93^{b}	44.39^{ab}
15 g *Trichoderma*	44.70^{b}	42.58^{abcd}
15 g *Trichoderma* + CaO (6 t ha^{-1})	47.39^{a}	43.53^{abc}
20 g *Trichoderma*	45.59^{b}	44.83^{ab}
20 g *Trichoderma* + CaO (6 t ha^{-1})	47.91^{a}	46.48^{a}
Fungicide (Flusulfamide) (300 kg ha^{-1})	46.87^{a}	45.64^{a}
Lime (CaO) alone (6 t ha^{-1})	44.58^{b}	42.51^{abcd}
Triple 14 + Urea and CaO (Farmers Practice)	43.63^{bc}	41.66^{abcde}

Means with the same letter in a column are not significantly different at 5% level of DMRT

Marketable Yield

The highest marketable yield was obtained from plants administered with 20 g *Trichoderma* + CaO 6 t ha$_{-1}$ but not significantly from those administered with 20 g *Trichoderma* alone (Table 6). These two treatments had significantly higher yield from the rest of the treatments.

The difference in yield is attributed to better roots with lesser clubroot infection that allowed the cabbage to develop bigger heads. Application of 20 g *Trichoderma* alone resulted in a lower marketable yield of 8.3 t ha$_{-1}$ from 9.5 t ha$_{-1}$., a difference of 1.2 kg. Such result proves the beneficial contribution of *Trichoderma* in improving crop yield. Plants not treated with *Trichoderma* such as farmers practice and plants applied with lime alone had a lower mean marketable yield of 6.5 and 6.2 t ha$_{-1}$.

Table 6. Mean Marketable Yield (t ha-1) at harvest as affected by the application of *Trichoderma* KA

TREATMENTS	MEAN MARKETABLE YIELD
10 g *Trichoderma*	6.6[c]
10 g *Trichoderma* + CaO (6 t ha^{-1})	7.6[b]
15 g *Trichoderma*	6.5[c]
15 g *Trichoderma* + CaO (6 t ha^{-1})	7.3[b]
20 g *Trichoderma*	8.3[a]
20 g *Trichoderma* + CaO (6 t ha^{-1})	9.5[a]
Fungicide (Flusulfamide) (300 kg ha^{-1})	7.5[b]
Lime (CaO) alone ((6 t ha^{-1})	6.2[c]
Triple 14 + Urea and CaO (Farmers Practice)	6.5[c]

Means with the same letter in a column are not significantly different at 5% level of DMRT

CONCLUSIONS AND RECOMMENDATION

The local isolate *Trichoderma* KA is a potential biological control for clubroot (*Plasmodiophora brassicae*) in cabbage.

Seedbed and field application of the biocontrol agent two weeks before sowing the seeds and transplanting the seedlings reduces the clubroot severity and infection and provided a comparable control of 80.8
5. control to that of the 82.6 % control from the fungicide flusulfamide.

Cabbage had improved fresh and dry root weight and resulted in better yield. Plants that receive *Trichoderma* are heavier in weight.

Performance of *Trichoderma* as biological control and as biofertilizer is enhanced both in seed bed and field plots with the addition of lime.

Trichoderma KA can be used in managing clubroot in organic production of crucifers.

It can also be used in integrated disease management also referred to as good agriculture practice (GAP) to address similar problem in crucifers.

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