CHAPTER - ONE

INTRODUCTION

1.1 Clubroot Diseases

1.1.1 General

Clubroot is an infectious disease of plants of the Brassicaceae family caused by the obligate biotrophic protist *Plasmodiophora brassicae* Woronin. It is an obligate endoparasite in the roots of cruciferous plants, attacking both cultivated as well as wild members. The disease was first reported in the United States in 1852 (Karling, 1968). It was first recognized in Spain in the 15th century, and then it was recorded in England and in Scotland. In the middle of 18th century, the disease spread to most of the European countries. Woronin, a Russian researcher identified the pathogen and named as *Plasmodiophora brassicae* in 1875 (Hirai, 2006).

Historically, *P. brassicae* was included in the group of primitive fungi, but it is now grouped into the Protista and the phylum Plasmodiophoromycota (Braselton, 1995).

1.1.1.1 Symptoms of Clubroot

The underground parts show distinct symptoms and the above ground part show the symptom when the disease is sufficiently progressed (Woronin,1978 *cit.* Walker, 1957); Chupp 1917 *cit.* Walker, 1957). Roots are hypertrophied characterized by the formation of swollen, elongated areas on main root and developing rootlets. These thickened areas are malformed, knobby to club-shaped. The aboveground symptoms are stunted growth and a droopy condition, followed by wilting and dying (Chupp and Sherf, 1960; Walker, 1952).

It is very difficult to distinguish between the infected and healthy plants if their above-ground parts are only observed. The infected plants show following symptoms (Sharma, 1998).

- The first symptom appears on the underground part.
- Wilting of the leaves is above ground visible symptom.
- Plants appear yellow, stunted and show retardation in growth.

- Roots are hypertrophied and sometimes become 10-12 times enlarged to form club-shaped malformations.
- Infected root hairs are also hypertrophied. Their tips are also expanded and form club-shaped swellings. In severe infection the expanded tips of root hairs become lobed and branched. Structures like root buds are also formed.

1.1.1.2 Life Cycle

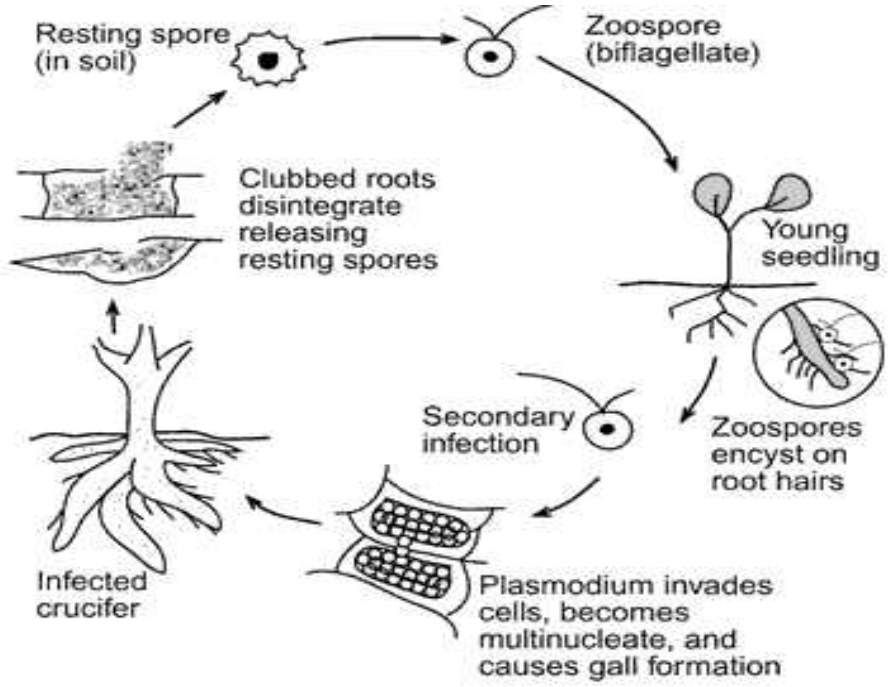
The Life cycle of the soil borne pathogen *Plasmodiophora brassicae* starts from its resting spores lying in soil or in the diseased plant debris (Crute, 1986; Lawrece, 1995) . The life cycle of starts with the germination of a primary zoospore from a haploid resting spore in the soil . In the presence of roots of a susceptible host, resting spores of the fungus germinate to produce motile zoospores .The zoospore attaches to a root hair and injects its cell contents into the host cell (Trembly *et al.*, 2005). Zoospore can also penetrate hosts at wound sites or underground stem through leaf scars (Toit, 1990).

The development of *P. brassicae* in root hairs into zoosporangia has also been observed in non-crucifers, including some monocotyledons. From the zoosporangia, haploid secondary zoospores are released. Naiki *et al*. (1987) showed that secondary zoospores again can infect root hairs, which results in rapid asexual propagation of the pathogen. Pathogenesis starts with the secondary zoospores. Two zoospores fuse, resulting in dikaryotic zoospores. However, genetically uniform single-spore isolates can complete the disease cycle, implying that fusion of zoospores is not necessary, or that homothallic genotypes of *P. brassicae* exist. After infection of the root cortex, the pathogen exists as intracellular, multinucleate plasmodia (Narisawa *et al*., 1996).

It was observed that plasmodia isolated in the infected callus tissue, did not penetrate the host cell wall. They postulated that the spread of the pathogen occurs mainly by stimulated division of infected cells, a process which results in the formation of the clubs. The enhanced cell division is thought to be stimulated by the elevated concentrations of cytokines and auxins. The auxins are presumably derived from indole glucosinolates,

normally present in roots of crucifers, due to the presence of intracellular *P. brassicae* plasmodia (Butcher *et al*., 1974). Later in the development, the haploid nuclei in multinucleate plasmodia fuse in pairs. After meiosis the newly formed diploid nuclei develop into haploid resting spores which are released into the soil when the clubbed roots decay. During this second stage pathogenesis, as many as 10 resting spores per plant can be produced (Voorrips, 1996).

Severe clubroot symptoms were observed on very young plants on the taproot in the production field suggesting that most of the infections are likely to occur on the transplant nursery beds (Correll, 2006). Within the infected plant roots, the organism develops rapidly, causing an increase in the number and size of cells, which results in "clubbing." During the development of the organism in the plant, new zoospores are produced (Fig: 1). these are capable of infecting the same plant or adjacent plants and thus, repeating the cycle. Eventually, resting spores are formed within the diseased plant tissue, and these are released into the soil when the plant roots disintegrate (Garbeva *et al.*, 2004).



(Source: Donald, 2006)

Fig:1. Life cycle of *Plasmodiophora brassicae*, the pathogen that causes clubroot

1.1.1.3 Host range

The disease is restricted mainly to members of the cruciferous family, both cultivated and weeds, and to a few other plants. Cabbage, Chinese cabbage, Brussels sprouts, some turnips, wormseed mustard, and some species of candytufts are very susceptible while kohlrabi, kale, cauliflower, collards, broccoli, rutabaga, seakale, some turnips and radishes, and some species of candytuft are susceptible and rape (canola), black mustard, some turnip and radish varieties, and tumble mustard are moderately susceptible (Averre, 2000).

In some 'non-hosts' for *Plasmodiophora brassicae,* at least the first part of the life cycle, the root hair phase, can take place (Ludwig-Muller, 1999). In fact, the observation of sporangia in the root hairs was the reason why they were first suggested to influence *P. brassicae* germination. Also with the definition of a host used by Wallker (1969) – 'the plant that gets diseased from presence of a parasitic organism' – the term 'non-host' is misleading since the pathogen can cause disease in some of the plants, at least to some extent. As shown by Ludwig-Müller (1999), *P. brassicae* can even use some of these plants for production of viable resting spores, although only a limited extent. Therefore, the plants are poor hosts rather than non-hosts, and these plants might even be of importance for *P. brassicae* to keep a small population viable in the absence of better hosts (Ristano and Gumpertz, 2000).

Table: 1 Some cruciferous weeds susceptible to infection by *P. brassicae*.

S.N	Common name	Botanical name
1	Bird rape	*Brassica campestris* L.
2	Wild turnip	*B. tournefortii.* Gouan
3	Shepherd's purse	*Capsella bursa-pastoris* (L.)Medikus
4	Wild radish	*Raphanus raphanistrum* L.
5	Turnip weed	*Raphanus rugosum* (L). All
6	Charlock	*Sinapsis arvensis* L.

Source: Donald 2006

Table: 2 Non-cruciferous plants susceptible to infection by *Plasmodiophora brassicae*

S.N	Common name	Botanical name
1	Cocksfoot	*Dactylis glomerata* L.
2	Strawberry	*Fragaria sp.*
3	Yorkshire fog grass	*Holcus lanatus* L.
4	Stock	*Matthiola incana*

Source: Donald, 2006

Table 3 Non-susceptible cruciferous weeds susceptible to infection by *Plasmodiophora brassicae*

SN	Common name	Botanical name
1	Hoary cress	*Cardaria draba* (L.) Desv.
2	Lesser swine cress	*Coronopus didymus* (L.) Sm

Source: Donald, 2006

1.1.1.4 Survival of *Plasmodiophora brassicae*

Decaying clubs release a large number of newly formed resting spores of *P. brassicae* in the soil, which can remain infectious for periods up to 15 years (Wallenhammar, 1996). This extended survival has been explained by a consequential dormancy of the resting spores. Spore germination is expected to be induced by specific substances excreted from host plant roots (MacFarlane, 1970).It has been observed, however, that germination occurs also in the absence of host plant roots, and that it can be stimulated by various environmental factors (Donald, 2006).

The protected life of *P. brassicae* in the soil and inside plant roots makes it difficult to control, and fungicides do not completely control *P. brassicae* (Karling, 1968). In the absence of a host, *P. brassicae* survives as haploid resting spores with a diameter of 3-5 µm. The resting spore is a very resistant structure. Its cell wall (including membrane) consists of approximately 25% chitin, 2.5% other carbohydrates, 34% protein and 18% lipid (Moxham and Buczacki, 1983). Germination of resting spores results in liberation of biflagellate zoospores (primary zoospores). The frequency of germination increases with spore maturity, and is enhanced by increased humidity and temperature, reduced by

alkaline pH, and varies with certain inorganic ions in the soil (Macfarlane, 1970; Takahashi, 1994a). In contrast to the thick-walled resting spores, the zoospores are sensitive to different kind of environmental stress. Zoospores with no access to a host plant are considered to survive for only a short period of time (Takahashi, 1994b).

When a zoospore finds its host, it attaches to the root hair and injects its cell contents into the host cell where it develops into a multinucleate plasmodium and later into a multitude of uninucleate zoosporangia from which haploid secondary zoospores are released. The secondary zoospores can again infect root hairs, or infect the root cortex (Ingram and Tommerup, 1972; Mithen and Magrath, 1992). Before infection of cortex, two zoospores may fuse, resulting in a dicaryotic zoospore. It is not known whether this fusion is necessary or whether different mating types of *P. brassicae* exist (Voorrips, 1996).
In the root cortex and stele, *P. brassicae* forms intra-cellular multinucleate plasmodia that stimulate the invaded host cells and adjacent cells to grow and divide through elevated concentrations of cytokinins and auxins (Siemens *et al.,* 2002). This leads to the formation of the characteristic galls. During each infection, host DNA sequences are incorporated into the pathogen genome (Bryngelsson *et al.,* 1998). Later, the haploid nuclei in multinucleate plasmodia fuse in pairs. After meiosis, the diploid nuclei develop into haploid resting spores, which after degradation of the galls are released into the soil (Ingram and Tommerup, 1972; Wakeham and White, 1996). As many as 109 spores per gram of infected root have been recorded (Horiuchi and Hori, 1980; Siemens *et al.,* 2002). *P. brassicae* is reported from Brassica growing areas worldwide.

The level of pathogen success and the pathogen's ability to cause disease within a certain soil has led to the concept of soil suppressiveness to the pathogen or to the disease. Characteristics of agricultural soils make them more or less suppressive or conducive to different soil-borne plant pathogens. Some of these characteristics are determined by indigenous factors connected to soil type or climate, while others are influenced by cultural practices (Höper and Alabouvette, 1996). It has been suggested that *P. brassicae* resting spores have the ability to recognize host plant roots and that much of the germination is a specific response to the presence of host plants (Macfarlane, 1970).

1.1.1.5. Prejudicing factors

Prejudicing factors play an important role in the epidemics of the disease (Marshner *et al.,* 2004). Infection by *P. brassicae* depends on high soil moisture and disease development is favored by soil temperatures between 20 and 25^0C, although infection can also occur at temperatures as low as 12^0C (Timila, 2006). Disease development is highly favored in acidic soil. The disease can occur in temperature range of 12 - 27^0C but the optimum temperature for the growth of *P. brassicae* is 25^0C (Takahiro *et al.,* 2006).

Clubroot is more prevalent on poorly-drained soils, particularly low-lying areas. Clubroot is more pronounced in acidic soils (pH<7.0), but at high concentration level of the spore, the disease can develop in alkaline soils (pH>7.0) too. Crop losses are severe in warm-moist soils (generally between Octobers to April) which are heavily infested with *P. brassicae* (Donald, 2006). Although clubroot has been found in soil exhibiting a wide pH range from 4.5-8.1, the disease is primarily associated with acid soils (Trembly *et al.,* 2005).

1. 1.1.6 Inoculum density

The amount of Inoculum is one of the important attributes in inducing the clubroot disease (Strelkov *et al*., 2006; Narisawa *et al*., 2005). Generally, the inoculums for glasshouse tests is prepared by macerating fresh or frozen clubs, the swollen roots characteristic of the disease, in water using an electric blender. Another common inoculation method is all variations of the root dip slurry or pipette methods. After inoculation the plants are grown for six to eight weeks at high soil moisture. The symptoms are generally assessed visually and classified in grades. In most studies rather high inoculums densities have been used. In his review concerning the root dip and slurry methods, Dixon (l976) asserted that the inoculums densities used by different researchers varied from 1 to 10 resting spores per plant. Voorrips and Visser (l993) applied 2-10 spores per plant using the pipette method, and Rod and Robak (l994) considered 105 -108 spores per plant necessary for consistent results using a similar test method. It has long been recognized that high inoculums densities are required to ensure consistent symptom development on susceptible plants under varying environment.

In the experiments, inoculations were performed with varying numbers of resting spores per plant. The number of resting spores available per plant appeared to be most precisely controlled with the pipette inoculation method which was therefore utilized in this study conditions (Williams, 1996). Resting spore, which germinate and form zoospores as invades of host plants under favorable conditions, represents the only source of inoculums in natural infection (Yamuda, 2001).

1.1.1.7 Effects of plant species on *Plasmodiophora brassicae*

The effect of plants on soil microbial communities varies with the soil type and the combination of soil type and plant species (Roudriguez and Redman, 1997). Differences in root exudates amounts and composition are likely to affect community structure since microbial species differ in their ability to metabolize and compete for different carbon sources (Marschner and Yang, 2004).

Certain substances may have an inhibitory effect on soil microorganisms, and sometimes on the functioning of the whole microbial community (Yin *et al.,* 2004). Root exudates may also act as messengers that communicate interactions between roots and soil organisms, sometimes by mimicking different signaling molecules (Walker *et al.,* 2003). Similarly, other soil microorganisms, soil-borne plant pathogens as *P. brassicae* may be selectively influenced by plant species grown, directly by different substances found in root exudates, or indirectly through interactions with other soil microorganisms. Such effects of plants on pathogens have been included in the concept of induced soil suppressive ness, which has sometimes been correlated to specific groups of soil microorganisms but is mostly poorly understood because of the complexity of the microbial community (Garbeva *et al.,* 2004).

1.1.2 Clubroot Diseases in Nepalese Perspectives

Cultivation of *Brassica* vegetables has the highest potential for generating income among more traditional rice and maize farmers in Nepal. Among brassica vegetables, the most important are cauliflower (*Brassica oleracea* var.*botrytis*) and cabbage (*B. oleracea* var.*capitata*).The disease is potentially a destructive and particularly prevalent in the

temperate region but is very often known to occur in tropical region as well. In Nepal it was thought to be transmitted from India (Shrestha, 1997). In Nepal, for the first time clubroot samples of broccoli, knolkhol from one of the farmers of Katmandu was received at Plant Pathology Division in 1993 (PPD, 1994). Then the disease outbreak was observed in Bhaktapur. In the first year, the disease appeared in 5-7plants in cauliflower and it slowly spread to different fields of that area in the second year. Severe and wide spread epidemics have been observed since 2004 in Bhaktapur, Kathmandu, Lalitpur, Makawanpur districts and in Palung valley (Timila *et al*., 2008). Many cauliflower fields in these areas have had as much as 100 percent yield loss between 2004 and 2006 with an estimated 40 percent overall loss from clubroot. Timila *et al*, (2008) estimated cauliflower production was reduced from 5-6 metric tons per household (1500 square meters) prior to 2004 to less than 300 kg per household in 2004 and beyond in Palung. The economic loss in this area alone was estimated at USD 1.4 million in 2004 and 2005.

Examination of different nurseries indicated that more than 80% of the seedlings had symptoms of the clubroot disease at the time of transplanting. The loss varied from field to field, i.e. stages of crops, severity of the disease and also from farmers to farmers. Recently, a loss of US $ 1372 thousands was recorded within past three years due to the clubroot diseases in Palung valley, which is one of the most potential vegetable growing areas in Nepal (The Gorkhapatra, 2061). The disease incidence and severity were found higher in August/September than February transplanted crop (PPD, 2007). Up to 100%crop loss was estimated in some pockets of Bhaktapur (PPD, 2008).

Examination of transplant nurseries indicated that frequently more than 80 percent of the seedlings have symptoms of clubroot at the time of transplanting. Soil samples from the production areas indicated that the sandy loam soils were predominately acidic (pH range of 4.2 to 7.2 with more than 90 percent below 6.0). Now the disease has been recorded also in Ilam, Nuwakot, Dhading, Kavrepalanchok and Gorkha districts (Timila, 2006). The spread of the disease has been through rooted leafy vegetables sold in the local market. The disease could cause yield loss of 27-81% in the total bio-mass and 18-87% in curd yield of cauliflower.

1.2 Objectives and Justification

1.2.1 Objectives

The main objective of the study was to study the clubroot diseases in some farms of some villages of Bhaktapur and Kavreplanchok districts of Central Nepal. The specific objectives are:

1. To know the clubroot diseases severity in the study area.
2. To assess the damages caused by clubroot disease on cruciferous crops
3. To know the existing knowledge and management measures to control clubroot disease

1.2.2 Justification

Nepal has expressed its commitment to develop a national strategy for conservation and sustainable use of biological resources along with agricultural products. So, it needs to have detailed information and knowledge about its existing scenario in agriculture products and difficulties with plant pathogens. Very few studies related to Clubroot diseases in comparison with other diseases are found in Nepal. Bhaktapur and Kavre district deserves a great fame for adopting modern agricultural technologies and practices from the very beginning. Vegetable cultivation is a matter of pride for farming community in these districts. Heavy loss in cabbage and cauliflower due to clubroot disease is common in these sites. Therefore, present study will be helpful for adding up the knowledge upon the clubroot and their social and scientific parameters.

CHAPTER: TWO

LITERATURE REVIEW

Woronin a Russian researcher identified the pathogen and named as *Plasmodiophora brassicae* in 1875.He studied the structure and nature of the pathogen. He also described the major symptoms caused by the clubroot disease on the affected plants. (Woronin, 1978 *cit.* Chupp, 1917).

Larson and Walker (1934) had studied on the soil treatment in relation to clubroot of cabbage. Field treatments of Carrington silty clay loam and Clyde silty clay loam with sufficient Ca(OH)2, CaCO3 or MgCO3 to raise the reaction of the soil to pH 7.1 and above did not generally inhibit clubroot development, but the treatment was completely effective in preventing infection when the same soils were removed to the greenhouse. High or low relatively constant soil moisture did not change the degree of inhibition under greenhouse conditions. Fluctuation of soil moisture and forced aeration, however, did permit varying degrees of infection in soil. Low fluctuating soil moisture may be an important factor in limiting the effectiveness of lime as a clubroot inhibitor in the field.

Potts et al (1935) had experimented on the finger and toe-disease (*Plasmodiophora brassicae*). Turnips grown in pots were not affected with the finger-and-toe disease when the pH of the soil was maintained above 7.0 by frequent applications of Ca (OH) 2, Na2CO3 or KOH. They also observed that AcOH, H2SO4 and H3PO4 favored the development of the disease. The disease did not develop on turnips grown in soils containing 30% or more of CaCO3 in the form of chalk, but it did develop when the CaCO3 content was reduced to 10% or less. A high incidence of the disease occurred in all cases where horse manure was added to the soil-CaCO3 mixtures. Soils naturally rich in Ca were much less subject to the disease than were those deficient in Ca. In pot experiments with mustard and candytuft, the disease was markedly reduced by application of KNO3, NaNO3 or Ca (NO3)2 at the rate of 1 ton/acre. A moist soil was favorable to the development of the disease. The presence of organic matter in the soil is

not necessary for the development of finger-and-toe disease, though it may encourage the disease by enabling the soil to retain its moisture.

Haenseler (1937) had studied on the control of clubroot of crucifers. Metallic Hg mixed with fertilizer and applied 2 in. below and 2 in. to the side of the seed at rates equivalent to 8.3 and 16.6 lb. Hg per acre of 24 in. rows reduced club-root infection to 10 and 5%, respectively, as compared with 17% when fertilizer alone was used.

Walker (1957) had studied on the different factors that influence the clubroot disease. Clubroot disease was found in soil exhibiting pH ranges 4.5-8.1. The disease primarily associated with acidic soil. Soil was found to infest with *Plasmodiophora brassicae* for 10 years or longer without the presence of host plants. The fungus is one of the most persistent soil invaders and crop rotation was of little significant in control.

Chupp and Sherf (1960) had studied on the external visible symptoms of the plants by the clubroot disease .The disease was characterized by the formation of swollen elongated areas on main root and developing rootlets. These thickened areas were malformed, knobby to club-shaped. The aboveground symptoms were stunted growth and a droopy condition, followed by wilting and dying. Leaves of the infected plants assumed a pale-green or yellowish color. Complete clubbing of main and lateral roots. The clubby roots consist of host's cells containing spores of the fungus. The infection was through the root hairs.

Dekhuijzen (1979) had done electron microscopic studies on the root hairs and cortex of a susceptible and a resistant variety of *Brassica campestris* infected with *Plasmodiophora brassicae*.The cortex of the roots of a susceptible and a resistant variety of*Brassica campestris* var.*rapa* infected with sterile resting spores of *Plasmodiophora brassicae* from senescent callus was studied at a stage prior to disease symptom development. Electron micrographs show the presence of amoeboid structures within the cortical cells of the susceptible variety 10 days after inoculation. Cell wall perforations, hypertrophied host cell nuclei, nucleoli and broken tonoplasts were frequently found in the susceptible

variety. It has been concluded that amoeboid structures of the parasite penetrate the cell wall and disrupt the cortical cells. Electron micrographs of the resistant variety show the presence of zoosporangia with secondary zoospores in the root hairs nine days after inoculation. Two to four days later a large number of dead host cells can be observed in the outer cortical layer of the resistant variety, whereas no apparent changes are found in the inner cortex. The results suggest the occurrence of a hypersensitive host reaction which terminates further growth of *Plasmodiophora brassicae*.

Porter *et al.,* (1991) had studied on Soil solarisation combined with low rates of soil fumigants controls clubroot of cauliflowers, caused by *Plasmodiophora brassicae* Woron. The effect of solarisation combined with low rates of soil fumigants on the severity of clubroot and yield of cauliflowers was determined at 2 locations in southern Victoria. The effectiveness of treatments was shown to be dependent on location, on the type, water content and temperature of soil, and on the population density of *Plasmodiophora brassicae*. Yields were reduced depending upon the disease severity, usually within 60 days after transplanting. Propagules of *P. brassicae* could survive for more than 28 days in ovens at 45^0C in dry soil but died within 14 days at 40^0C in moist soil. At Werribee in 1985 on a red brown earth, solarisation combined with dazomet (100 kg dazomet/ha) gave significantly better control than either treatment alone. This treatment reduced *P. brassicae* in the 0-10 cm layer, reduced the disease rating from 2.7 to 0.9 (0-3), and increased yield from 2.4 to 47 t/ha compared with controlsThese treatments were more effective than solarisation and dazomet used alone or in combination.

Voorrips & Visser (1993) had studied on the examination of resistance to clubroot in accessions of *Brassica oleracea* using a glasshouse seedling test. A glasshouse test was elaborated for assessing large numbers of seedlings of *Brassica oleracea* for resistance to clubroot, a disease caused by the fungus *Plasmodiophora brassicae*. The method offers good control of inoculum density per plant, and requires 6–7 weeks from sowing. The results from the glasshouse test correlated well with field test results. With this method, 71 accessions of *B. oleracea* reported to carry resistance to clubroot, and one susceptible

control cultivar were tested with a Dutch clubroot isolate. High levels of resistance were found in several accessions of cabbage, broccoli and curly kale. F1-populations of resistant cabbage or curly kale × susceptible cabbage were fully susceptible, indicating recessive inheritance of resistance in all cases.

Morgner & Sacristan (1995) had studied on the quatitative determination of colonization by *Plasmodiophora brassicae* in *Brassica napus* and *Brassica oleracea.* For the study of plant pathogen interactions especially with combination with obligatory pathogens with different demands for the target tissue-root versus leaves.it may be useful for crucifer breeders in situations of parallel testing of the same plant individual with numerous races of the *Plasmodiophora* and *Hyaloperenospora*, substantially diminishing the risk of the accidental contamination . however , the smaller the *Plasmodiophora* galls in induced roots hampers the indexing fo the disease severity and calls for more objectives methods of evaluation such as histochemical staining.

Voorrips (1996) conducted the research on the clubroot in the cole crops and studied the interaction between *Plasmodiophora brassicae* and *Brassica oleracea*. It is an important disease, affecting an estimated 10 % of the total cultured area world-wide. The potential of cultural practices to reduce crop losses due to clubroot are limited, and chemical treatments to control the fungus are either banned due to environmental regulations or are not cost effective. Breeding of resistant cultivars therefore is an interesting alternative. Researcher addressed some aspects of the *P. brassicae - B. oleracea* interaction associated with resistance breeding. A seedling test for clubroot resistance was developed. Symptom development in this test was shown to correlate well with symptom development in the field situation. The seedling test was used to identify *B. oleracea* accessions resistant to a Dutch field isolate of clubroot.

Timila and Shrestha (2000) had studied on the integrated management of clubroot disease of cauliflower. Clubroot caused by *Plasmodiophora brassicae Woronin* was appeared to be an economically important disease of cruciferous vegetables crops, especially cauliflower of Kathmandu valley. Disease incidence was observed up to 100%. Since the

farmers do not like to follow crop rotation with other non-host crops, a trial was conducted during 1998/99 and 1999/2000 to find out the control measures in naturally infested field at Bansathali, Balaju, Kathamandu. Eight different treatments were used including control.Among the treatments, lime amendments at the rate of 3 t/h and seedling dipped in benomyl (0.2%) were found effective to reduce disease incidence and it's severity. The disease incidence in lime amendments and Benlate treated plots were 4.5% and 2.0% in 1998/99 and 29.9% and 16.6% in 1999/2000 respectively. The control plots showed 30.4% and 49.9% respectively. The biological agent *Trachoderma harzianum* and garlic extract also found to suppress disease incidence and severity of club. The incidence of disease in *T.harzianum* applied plot and garlic extract treatment were 17.74% and 13.24% in 1998/99 and 8.3% and 23.3% in 1999/2000 respectively. Marketable curd yield was also found higher in those treatments than in control.

Kobayashi *et al.*, (2002) studied the control of soil-borne clubroot disease of cruciferous plants by epoxydon from *Phoma glomerata*. Culture broth from an isolate of *P. glomerata* from the leaves of Viola sp., controlled the soil borne pathogen *P. brassicae* which causes clubroot disease of cruciferous plants. This effect was caused by epoxydon. Although this substance was known to have antitumour activity, phytotoxicity and antiauxin activity, no plant disease reduction had been reported previously. Epoxydon possessed neither strong antimicrobial activity nor did it induce acquired resistance. It protected crucifers from clubroot disease at 250 $\mu g\, mL^{-1}$ following addition to the soil. Several antiauxins were tested for similar properties resulting in the suppression of clubroot disease and one, 2,3,5-triiodobenzoic acid, was effective at 10 $\mu g\, mL^{-1}$. Clubroot reduction by epoxydon may result from antiauxin activity. This research opens opportunities for a new group of agrochemicals.

Timila and Shrestha,(2003) discussed about the integrated management of clubroot disease of cauliflower. Field research conducted at the farmer's field (Banasthali) showed the seedling treatment with Benlate reduced disease incidence and severity of clubroot in cauliflower compared to control.

Hanna (2005) had studied on the persistence of *Plasmodiophora brassicae*. Researcher found that, it has tolerant resting spores that permit survival in the absence of a host plant. The resting spores are expected to germinate when triggered by specific substances excreted from host plant roots, but they also respond to other clues. As the zoospores emerging at germination are sensitive and short-lived, stimulation of resting spore germination is a potential method for management of clubroot disease. Certain non-host plants have been found to increase resting spore germination. The effect of four non-host plants on *P. brassicae* was studied in laboratory-, greenhouse- and field studies. In the laboratory, root exudates solution of *Lolium perenne* (Rye grass) stimulated germination of resting spores more strongly than other non-host plants tested (*Allium porrum, Trifolium pratense*) or the host plant *Brassica rapa* var. *pekinensis* (Chinese cabbage). When grown in soil, however, no species-specific effect of any of the plants was observed on the persistence of *P. brassicae*. It has been claimed that resting spore germination increases in response to substances excreted from decaying plant material or to a general increase in soil biological activity.

Takahiro *et al.*, (2006) studied on the susceptibility of hairy root lines of *Brassica* species to *Plasmodiophora brassicae* and in an in vitro subculture system. To investigate the susceptibility of hairy root lines of *Brassica* species to *P. brassica*e , hairy roots were induced in a number of *Brassica* species with *Agrobacterium rhizogenes* . Turnip hairy root was highly susceptible to *P. brassicae* ; infection rates were high and large gall s formed . In contrast , the rates of root hair infection and gall formation on intact Brassica plants did not differ significantly from the control. To induce resting spore formation , turnip hairy roots were incubated at 15^0c, 20^0c or 25^0c after 3 weeks of incubation at 25^0c. The number and fresh mass of the galls per hairy root were higher and formation of resting spore was greatest after 7 week incubation at 20^0c .

Strelkov *et al.*, (2006) studied characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Clubroot caused by *P. brassicae* , was identified in a number of Cariola (*Brasicca napus*) fields in Central Alberta 2003 .To characterize the virulence of the pathogen in the province , field populations from a number of the

pathogen in the Edmonton region were tested on the two most widely used sets of differential hosts , those of P.H. Williums and European Clubroot differential (ECD) series. Populations from British Columbia and Ontario were included for comparison. While the reaction of some hosts could be clearly defined as either resistant or susceptibility, others showed intermediate diseases index scores .if disease indices of 0% -49% and 50%- 100% were regarded as resistant and susceptible respectively ,then populations from Alberta were classified as ECD 16/15/12 and 16/15/0 on the ECD set , or path types 3 and 5 respectively, on the hosts of Williums. The populations from British Columbia was classified as ECD 16/12/12 or pathotype 6 and the Ontario populations was classified as ECD 16/0/14 or path type 6. The Alberta populations were more virulent on the *B. napus* hosts than those from other provinces , perhaps a reflection of their Canola origin. In addition , 48 Canola cultivars including the 2004 prairie Canola variety trials were screened for resistance to a local populations of the pathogen and all appeared to be highly susceptible . If clubroot were to become more widely established in western Canada it could have a major negative impact on yields.

Pageau *et al*, (2006) studied on impact of clubroot on productivity and quality of Canola. Clubroot, a disease caused by *P. brassciae*, first appeared in Canola fields in Quebec in 1997. The objective of this project was to assess on the impact of a soil infested with *P. brassciae* on the productivity and grain quality of canola in 1998. 31 cultivars in total (23 of *B. napus* in 1998 or 25 of *B. napus* in 1999 and 6 of *B. rapa*) were evaluated. In 2000, 25 cultivars and two lines (70584 ey 70585) of *B. napus* and 6 cultivars of *B. rapa* were tested .These cultivars or lines were sown on a soil where *P. brassicae* ahd been prevalent for a few years and on another soil which had never been sown with a cruciferous crop before,. The soil infested with *P. brassicae* reduced Canola productivity. Grain yield losses were 80%, 91% and 85% in 1998,1999 and 2000 respectively, for the Argentine Cultivars (*B. napus*). In the Polish cultivars (*B. rapa*),yield losses were 69%,96% and 89% respectively, for the same years. Clubroot also reduced straw yield, oil content of the grain and the grain mass. As opposed to overall *B. napus* cultivars, two lines were not affected by the

disease. Since there is no registered Canola cultivar tolerant or resistant to clubroot in Quebec, Agronomic practices used to prevent or reduce the incidence of the disease should be encouraged.

Friberg *et al.,* (2006) had studied on usefulness of non-host plants in managing *Plasmodiophora brassicae.* Germination of resting spores of *P. brassicae*, causal agent of clubroot in crucifers, may be stimulated by certain non-host plants. Without a host plant to infect, such germination would lead to a reduced persistence of resting spores in the soil. The effect of four non host plants on *P. brassicae* was investigated in a 3-year field experiment and a 14-month glasshouse experiment. Three of the plant species used leek (*Allium porrum*), winter rye (*Secale cereale*) and perennial ryegrass (*Lolium perenne*), have been reported to stimulate resting spore germination, while the fourth, red clover (*Trifolium pratense*), does not. In the field experiment, none of the plant species reduced the concentration of *P. brassicae* in soils when tested with bioassay plants (Chinese cabbage, *Brassica rapa* var. *pekinensis*). In the glasshouse experiment, there was a lower disease level in all plant treatments compared with the plant-free control following incorporation and decomposition of plant roots. At this time, pH in the soils with plant treatments was higher than that in the control soil. There were no indications of a species-specific interaction between any of the nonhost plants investigated and *P. brassicae*, and it cannot be concluded that any of them would be useful in the sanitation of *P. brassicae* infested soils within short time periods.

Reiko *et al.,* (2007) conducted the research on clubroot and organic matter application. They found that , clubroot disease of the cruciferous plants caused by the soil-borne pathogen *Plasmodiophora brassicae* is difficult to control because pathogen survives for a long time in soil a s resting spores . Disease suppressive and conducive soils were found during the long –term experiment on the impact of organic matter application to arable fields and have been studied to clarify the biotic and a biotic factors involved in the disease suppression. The fact that a large amount of organic matter 400 t ha(-1) yr(-1) farmyard manure (FYM) or 100 t ha (yr(-1)) food factory sludge compost (PSC) , had been incorporated for more than 15 years in the suppressive soils and these soils showed higher pH and Ca concentration than the

disease conducive soil led us to hypothesize that an increase in soil pH due to the long-term incorporation of Ca-rich organic matter might be the primary cause of the disease suppression .

Siemens *et al.,* (2008) has studied on the monitoring expression of selected *P. brassicae* genes during clubroot development in *Arabidopsis thaliana*. The expression of 12 cDNAs from *P. brassicae,* among them two novel sequences, was determined during clubroot development on Arabidopsis thaliana. The aim was to find cDNAs expressed at distinct stages of pathogenesis. The relative amount of infection with active plasmodia could be estimated using PbActin cDNA as an internal standard. Two cDNAs, PbBrip9 and PbCC249, were strongly expressed at stages of disease development corresponding to the occurrence of sporulating plasmodia. Therefore, it should be possible in the future to find more cDNAs which could be used as markers for certain stages of clubroot development.

McDonald *et al.,* (2008) studied on temperature prior to harvest and severity on two Asian Brassica vegetables. Data from 17 different seedlings at the research station over 4 year were used to compare the relationships between disease incidence and DSI and weather conditions during crop development. Clubroot incidence and severity were highest for crops harvested in July and August and lowest in crops harvested in October. Mean air temperature during crop development ranged from 15 to 22^0C and were positively correlated with clubroot incidence and severity for both Pak Choy (r = 0.68) a flowering cabbage (r = 0.73). The strongest correlation occurred between air, temperatures and disease severity over the final 10d before clubroot damage in Asian *Brassica* vegetables could be minimized by seedling in early spring and late summer in areas infested with *P. brassicae.*

Timila *et al.,* (2008) studied on severe and widespread clubroot epidemics in Nepal. According to them, typical disease symptoms are widespread, and disease severity has been particularly severe in the Kathmandu Valley and Palung and Daman area of the Makwanpur District. Many cauliflower fields in these areas have had as much as 100% yield loss between 2004 and 2006 with an estimated 40% overall loss from clubroot.

Estimates from interviews with growers in the Palung production area during an intensive farmers' interaction program indicated that cauliflower production was reduced from 5 to 6 metric tons per household (1,500 m^2) prior to 2004 to <300 kg per household in 2004 and beyond. The economic loss in this area alone was estimated at $1.4 million in 2004 and 2005. Examination of transplant nurseries indicated that severity was high at the time of transplanting. Soil samples from throughout the production areas indicated that the sandy loam soils were predominately acidic (pH range of 4.2 to 7.2 with >90% below 6.0). Several management practices are being employed to reduce disease severity, including the use of clubroot resistant cultivars, raising the soil pH to >7.0 by using dolomitic lime, testing of the fungicide Krilaxyl (Nebijin) and biopesticide Sanjeevani (*Trichoderma viride*), and biofumigation and solarization of the nursery beds in an effort to reduce disease pressure on transplant material.

Plant Pathology Department, NARC (2008) reported the serious problems of Clubroot of Brassicas throughout Bhaktapur district Central Nepal. Research was carried out at Suryabinayak, Katunje in Bhaktapur district. Research concluded that Nebijin was the best treatment in controlling clubroot disease and in yield parameter followed by Derosal.

Siemens, *et al.,* (2009) studied on molecular biology of *Plasmodiophora brassicae*. Initially, molecular techniques were used to detect and distinguish *Plasmodiophora* pathotypes in soil. Meanwhile, chromosomes from 2.2 Mb to 680 kb are characterized and the total genome size is estimated to be approximately 20 Mb. Furthermore, the genomic gene structure and the cDNA structure of several genes have been revealed, and the expression of those genes has been linked to development of clubroot to some extent. In addition, the sequence data have reinforced the inclusion of the plasmodiophorids within the Cercozoa. The recent successes in molecular biology have produced new approaches for clubroot research.

CHAPTER: THREE

STUDY AREA

3.1 Background

The study was carried out at Tukucha Nala VDC of Kavreplanchok District and Sudal and Tathali VDCs of Bhaktapur District, Central Nepal.

3.2 Districts Overview

3.2.1 Bhaktapur

Among the various districts in Bagmati zone of Central developmental region, Bhaktapur is the one which extend from 27^{o}36' to 27^{o}44' North latitude and 85^{o}21' to 85^{o}32' East longitude having a total length of 16 km from east to west. Bhaktapur is the smallest district of Nepal covering the area of 138.46sq.km with its elevation ranging from 1300m above sea level to 2191m above sea level. The district experiences sub-tropical to temperate climate with an average annual rainfall of 56 mm. During summer season it reaches the maximum temperature of 32˚C, whereas during winter season it reaches a minimum temperature upto -2˚C (District Development committee, Bhaktapur).The district is surrounded by the Kathmandu in north-west, Lalitpur in south-west and Kavrepalanchok in south-east. It comprises of 2 metropolitan cities and 16 V.D.Cs. On the basis of the nature of land, it has been divided into two regions viz; Hilly region and Valley region which combinely contains 41,253 households with 2, 25,461 (male-1, 14,798 and female-1, 10,663) population. The population growth rate is 2.7% (CBS, 2002). The sites for this study were selected at Sudal and Tathali VDCs of Bhaktapur District.

3.2.2 Kavrepalanchok

Kavrepalanchok is also one of the district in Bagmati zone under the Central Developmental region which lies in between 85^{0} 24' to 85^{0} 49'E longitude, and 27^{0} 20' to 27^{0} 85'N latitude. Dhulikhel, the headquarter is 31 km far away towards east from Kathmandu. The mercury touches 28^{0}c at summer whereas in winter, it falls upto 9^{0}c. The district experiences sub-tropical to cool temperate climate with an average annual rainfall varying from 1300mm-2687mm depending on the sites. Ramechap and Dolakha lies in the east of the district and Sindhupalchowk in the north whereas Kathmandu, Bhaktapur and Lalitpur in the west and Sindhuli and Makwanpur in the south. The lowest

level of the district, Dolalghat is in 275m from the msl and the highest level is Bethanchok danda at the elevation of 3018m from the mean sea level. Covering an area of 1404.86 sq. km, this district is divided into 87 VDCs, and 3 metropolitan cities. The total population of the district is 385,672 comprising of male 88,947 and female 196,725. The population growth rate is 1.7% (CBS, 2002). The site for this study was selected at Tukucha Nala VDC of Kavreplanchok District.

Vegetable cultivation is a matter of pride for Bhaktapur and Kavrepalanchok districts. District deserves great fame for adopting modern agricultural technologies and practices from the very beginning. Agriculture is the main occupation of the people and 64.45% and 71.05% of them are engaged in agriculture in this district respectively. It is capable of fulfilling about 80% demand of vegetables of Kathmandu metropolitan city, Bhaktapur and Lalitpur sub-metropolitan city. Local farmers of Bhaktapur are dedicated and laborious farmers of the district since ancient time. It stands as a model district in the context of green revolution. Intensive farming system is most characteristic feature of these districts.

Regarding the agricultural problems, diseases and pest problem are most severe in these districts which have caused that high value crop in decreasing condition. Although Nepal Agricultural Research Council and various other institutions have developed various high yielding technologies focusing on technical aspects only and not considering the farm management, financial and marketing aspects.

CHAPTER - FOUR

MATERIALS AND METHODS

4.1 Methods of Data Collection

4.1.1 Secondary Data Collection

Secondary data were collected from intensive literatures review. Related secondary data were collected through the different authorities and research institutions.

4.1.2 Primary data collection

4.1.2.1Reconnaissance Survey

A preliminary survey was conducted prior to field observations. This survey was conducted in order to identify the general features and existing situation or the prevailing situation of the study area. Rapport building with the growers was a major objective of this survey. At the same time this survey was also aimed to identify key informants of the study. Locals were also informed about the objectives of this study to facilitate the primary data collection procedure.

4.1.2.2 Collection of Samples

The frequent filed visits (2 visits in a week) were conducted in three months duration (March - May 2009) in Tukucha Nala VDC of Kavreplanchok District and Sudal and Tathali VDCs of Bhaktapur District, Central Nepal (Plate: 1). For the ease of data interpretations and analysis, the visits were categorized into three broad visits i.e. first visit, second visit and third visit. First visit was considered total visits up to early seedling transplanting stage. Similarly second visit was considered within one month duration after first and last visit comprised total visits after second visit. Care was taken to cover all the major developmental stages of crucifers. Cauliflower and cabbage plantation sites were chosen for this study as they dominate off season crucifers' cultivation. Attempts were made to collect the information from other crucifers and alternate hosts also. In total 45 plots of 5mx5m size were established in three VDCs. Roots of seedling infected with clubroot and in some cases only with morphological aerial symptoms were counted in each plot in each visit (two visits in each week). Local farmer's perception and observatory methods were applied. While conducting field trips, local assistants among the farmers from each community were accompanied (Plate: 2).

4.1.2.3 Soil sampling

Twelve soil samples were collected from the experimental field, four from each VDCs. The samples were taken from the 15 cm depth by a soil tube auger from soil of the diseased and diseased free area (3 from soil of the diseased and 1 from diseased free area of plantation site). Different soil parameters like pH, organic matters, available Nitrogen, available Phosphorous, available Potassium, Cations exchange capacity like Calcium, Magnesium, Sodium and Texture of the collected soils were measured at Nature Lab (P.) Limited, Bhaktapur.

4.1.2.4 Focus Group Discussions and Questionnaires Survey

A group discussion was organized with local farmers and with the major growers identified during the reconnaissance survey to collect a variety of information and ideas. The household questionnaire was developed with input from the preliminary related works, experts, and dissertation supervisor. The questionnaire was pre-tested in five hhs together with local assistant. Thereafter, the final version of questionnaire was produced for use in the field (Annex-I). Seventy five households were selected for the study.

4.2 Data Processing and Analysis

Unnecessary detail and insignificant information have been eliminated by editing collected data. Data have been arranged in the tabular form according to the need of research content. Data analysis has been accomplished with the help of tabulation.

CHAPTER - FIVE

5. RESULTS

5.1 Scientific Findings

5.1.1 Diseases Occurrence

Data were recorded to find the total crucifer plants affected with clubroot disease in Tukucha Nala VDC of Kavreplanchok District and Sudal and Tathali VDCs of Bhaktapur District. 15 research plots of each $5m^2$ were established in vegetable farm of each village. Altogether 20 visits were made to record the data. From 15 plots of $5m^2$ (total area coverage =375 $m^{2)}$ area total numbers planted crucifers in early stage, plants affected with clubroot diseases and healthy plants till harvesting period were recorded simultaneously. Normally, it was found that local farmers transplanted 42-45 cabbage and cauliflowers seedlings in each research plot of $5m^{2.}$ They have almost similar practice that they cultivate each seedling in 20 cm (1 *haat*).

5.1.1.1 Clubroot disease at Tukucha Nala VDC

Result shows that among 663 plantlets planted in 15 plots 36, 22 and 8 plants were found infected in each category of first, second, and third visits respectively (Table: 5.1).This indicates 5.43%, 3.32% and 1.21% of infection on three respective visits. From the observation it was found that the minimum infection was observed in the plot no.3 which is 2.22% where as maximum infection was seen on the plot no.6 and 12 which was 14.29%. In total of 10.00% of the plants are found to be diseased in Tukucha Nala Study area. Result shows that the infection rate is high in early stage of plant development. Based on farmer's traditional knowledge on disease and its morphological symptoms, diseased plants were identified in each visit. The plantlets which farmers uprooted by themselves also recorded. Care was taken to gather the counting of all the infected plants. The awareness campaign was conducted to the relevant farmers likewise some compensation was paid to them. As shown in Table 5.1, the severity was observed to be declined in second and third visits respectively. This was due the fact that the infected plants in the first visit group were uprooted and burnt carefully. The less numbers of severity than expectation could be due to the off-season cultivation practice and may be due dry season. The discussion about it is made in discussion section.

Table 5.1: Severity of Clubroot disease at Tukucha Nala VDC at three major development stages of crucifers

Plot. No	No. of seedlings	First Visit	Second Visit	Last Visit	Total No. of diseased plants	Disease %	Mean Disease %
TN1	45	2	1	0	3	6.67	
TN2	46	3	1	1	5	10.87	
TN3	45	1	0	0	1	2.22	
TN4	43	3	1	0	4	9.30	
TN5	45	2	1	1	4	8.89	
TN6	42	3	2	1	6	14.29	
TN7	45	2	1	1	4	8.89	
TN8	43	3	2	0	5	11.63	
TN9	45	3	2	1	6	13.33	
TN10	42	2	2	1	5	11.90	
TN11	45	2	1	0	3	6.67	
TN12	42	3	2	1	6	14.29	
TN13	45	3	2	0	5	11.11	
TN14	45	3	2	1	6	13.33	
TN15	45	1	2	0	3	6.67	
	663	**36**	**22**	**8**	**66**		**10.00**

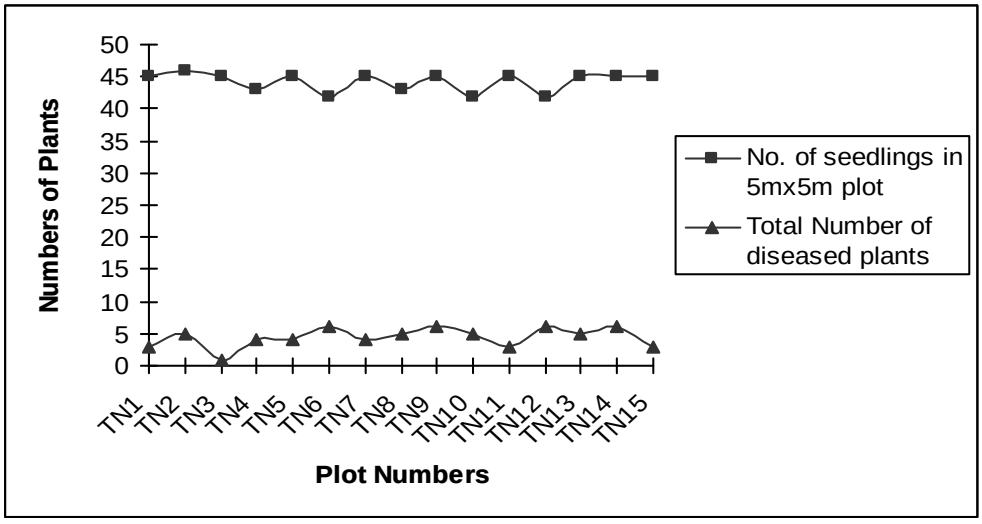


Fig.5.1. Severity of Clubroot disease at Tukucha Nala V.D.C.

5.1.1.2 Clubroot disease at Sudal VDC

Result shows that among 665 plantlets planted in 15 plots, 36, 27 and 8 i.e. 5.41%, 4.06% and 1.20% plants were found to be infected in each category of first, second, and third visit respectively (Table 5.2). The observations concluded that a total of the 10.69% infection was found in the study site. The mean disease incidence found in Sudal VDC was found comparatively greater than two other study sites. Maximum percentage of the infection was occurred in the second plot which was of 13.95% of infection on total plant of this plot in the Sudal VDC. And the minimum infection was seen on the tenth plot which was of 6.67%. The result of disease development is found to be similar with Tukucha Nala VDC.

Table 5.2: Severity of Clubroot disease at Sudal VDC

Plot. No	No. of seedlings	First Visit	Second Visit	Last Visit	Total No. of diseased plants	Disease %	Mean Disease %
S1	45	2	1	1	4	8.89	
S2	43	3	2	1	6	13.95	
S3	45	3	2	1	6	13.33	
S4	44	1	2	0	3	6.82	
S5	45	3	1	0	4	8.89	
S6	42	2	3	0	5	11.90	
S7	45	2	3	1	6	13.33	
S8	44	3	1	1	5	11.36	
S9	45	3	2	0	5	11.11	
S10	45	1	2	0	3	6.67	
S11	44	3	1	1	5	11.36	
S12	45	3	2	0	5	11.11	
S13	44	2	1	0	3	6.82	
S14	45	3	1	1	5	11.11	
S15	44	2	3	1	6	13.64	
	665	**36**	**27**	**8**	**71**		**10.69**

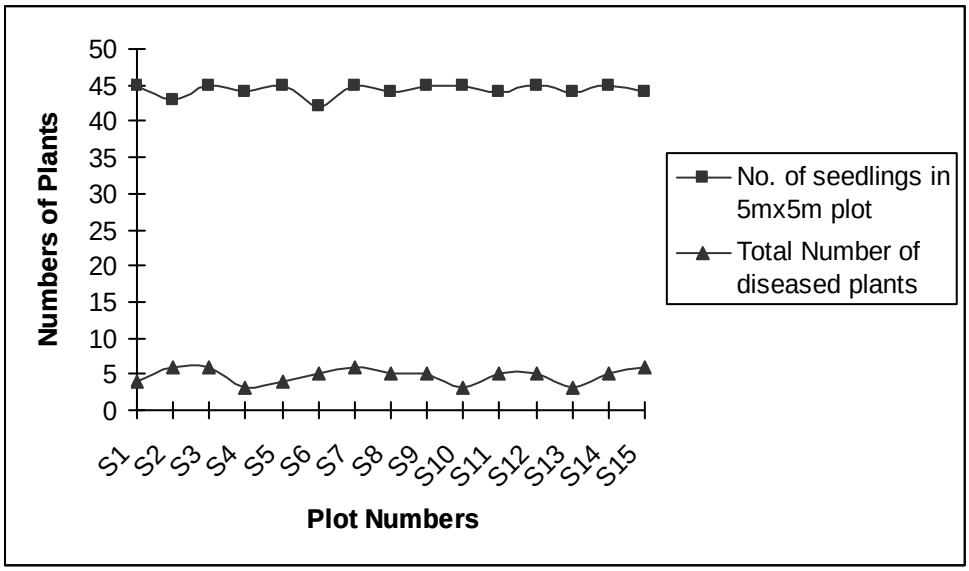


Fig.5.2. Severity of Clubroot disease at Sudal V.D.C.

5.1.1.3 Clubroot disease at Tathali VDC

Result shows that among 663 plantlets planted in 15 plots 37, 20 and 12 plants were infected in each category of first, second, and third visit respectively (Table 5.3) ,which was of 5.58%, 3.01% and 1.81% respectively of the total plant analyzed for the study. The experimental data showed that maximum infection occurred at the first visit and the decrease in infection level was seen on the second and third visits. This is due to fact that the diseased plant in each visit is uprooted with great care and it was destroyed by burning as done in other study areas. The overall infection of 10.45% was seen in this site. From the experimental findings, it was found that the maximum percentage of occurrence of disease was hold by plot no.9 which had the 16.67% of the infection and the minimum occurrence of this disease was found in the plot no.8,11,14 that covered 6.67% of disease incidence. This is presented on the graph below. As of observations made in Tukucha Nala and Sudal, the case was same for Tathali also. The severity was high in first visit and followed by second and third visits. As the ecological setting of Tukucha Nala, Sudal and Tathali is almost same, one could expect similar kind of observation.

Table: 5.3. Severity of Clubroot disease at Tathali VDC.

Plot. No	No. of seedlings	First Visit	Second Visit	Last Visit	Total No. of diseased plants	Disease %	Mean Disease %
T1	45	3	2	1	6	8.89	
T2	44	3	1	0	4	13.95	
T3	45	3	2	1	6	13.33	
T4	43	1	2	0	3	6.82	
T5	45	2	1	1	4	8.89	
T6	45	3	0	2	5	11.90	
T7	42	3	2	1	6	13.33	
T8	45	2	1	0	3	6.67	
T9	42	3	3	1	7	16.67	
T10	45	3	2	0	5	11.11	
T11	45	2	1	0	3	6.67	
T12	45	3	1	1	5	11.11	
T13	43	3	2	1	6	13.95	
T14	45	1	0	2	3	6.67	
T15	44	2	0	1	3	6.82	
	663	**37**	**20**	**12**	**69**		**10.45**

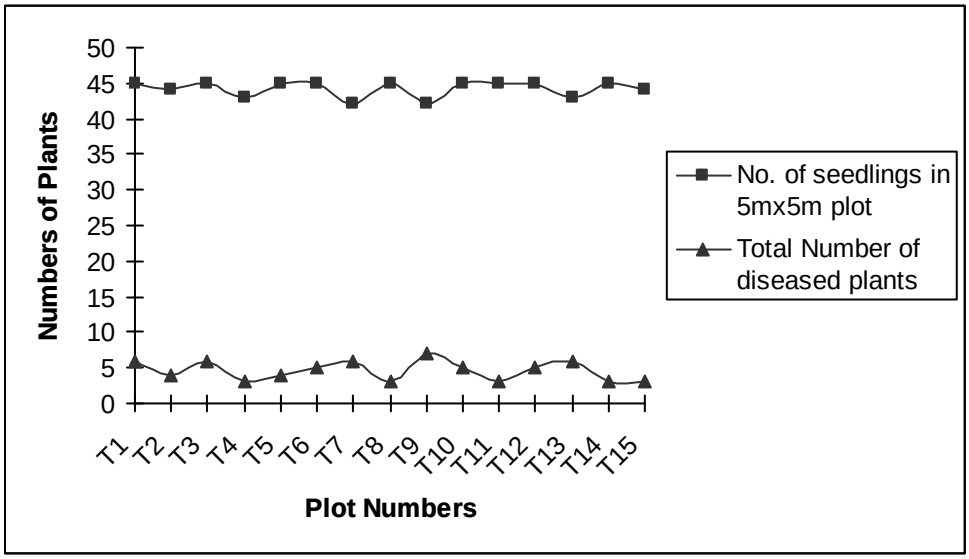


Fig.5.3. Severity of Clubroot disease at Tathali V.D.C.

In total from all the plots, 663, 665 and 663 seedlings were observed in Tathali, Sudal and Tukucha Nala VDC respectively.The research was carried out in the summer season where no water-logged situation promoting acidic nature to soil was present. But from the results it can be seen that the infection rate was high in each site. From the experiment, the infection found in Tukucha Nala V.D.C. is 10.00% and that of the Sudal and Tathali V.D.C. are 10.69% and 10.45% respectively which is too high for this season. Among

them 69, 71 and 66 plants were found with clubroots disease in Tathali, Sudal and Tukucha Nala respectively. This indicates the 89.55%, 89.31% and 90.00% of the cabbage and cauliflowers were found to be disease free till harvesting in three respective VDC's of the Bhaktapur and Kavreplanchok districts. So, a number of healthy plants remain till harvesting periods. Such healthy plants were 594, 594 and 597 respectively. As the observation methods were applied and farmers perception were incorporated, the plants which supposed to be diseases free may not be healthy plants, because the clubroot disease in those plants may be in initial stage of development. From this study it was found that disease intensity is high in Sudal VDC in comparison with other VDCs

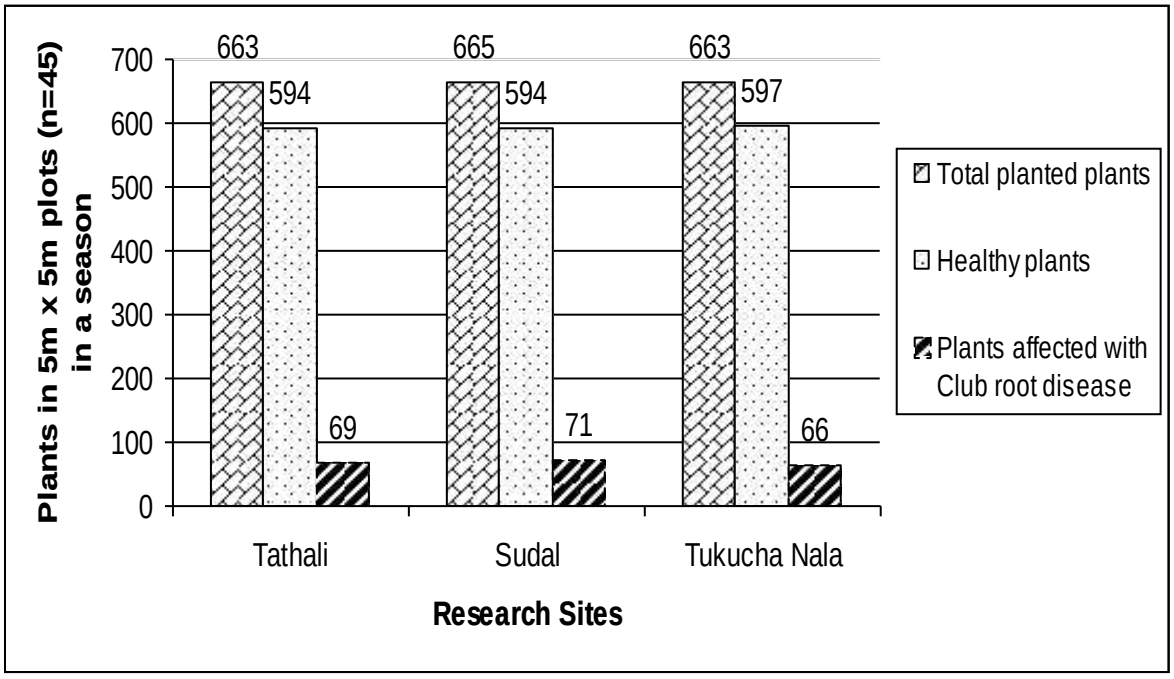


Fig: 5.4.Clubroot severity in research plots at different research sites

5.1.2 Soil Analysis

Special attention was made to find out the physical and chemical properties of soil samples collected from different research sites. Twelve soil samples were collected from the experimental field, four from each VDC. Among them 3 soil samples were collected from diseased prone areas and 1 from diseased free area of plantation site. Different soil parameters like pH, organic matters, available nitrogen, available phosphorous, available potassium, Cations exchange capacity like Calcium, Magnesium, Sodium and Texture of

the collected soils were measured. Detail findings of soil analysis are presented in Table 5.4.

Table: 5.4. Results of laboratory analysis of soil samples collected from research areas

S.N.	Parameters	Research Sites											
		Tukucha Nala				Sudal				Tathali			
		IS-1	IS-2	IS-3	DF	IS-1	IS-2	IS-3	DF	IS-1	IS-2	IS-3	DF
1	**pH**	5	5	4.5	6	4.5	5	4.5	6.5	4.5	5	5	6
2	**Organic Matter (%)**	18.3	18.3	15.5	18.3	19	16.3	15.5	17.3	14.5	15.5	17.3	16.3
3	**Nitrogen, (%)**	0.7	0.7	0.6	0.7	0.65	0.7	0.5	0.7	0.51	0.8	0.7	0.75
4	**Phosphorous, (µg/g)**	8.2	8.2	8.4	8.2	8.5	8.5	8.1	8.3	8.4	8.7	8.2	8.3
5	**Potassium, (µg/g)**	114	113.65	110.55	113.65	115.5	112.65	112.5	112.15	105.8	106.65	107.65	114.15
6	**CEC**	8	7	8	8	8	7	8	8	7	8	7	8
7	**Cations (mg/g)**												
	Calcium	1.01	1.02	1.12	1.01	1.11	1.01	1.15	1.11	1.12	1.01	1.05	1.01
	Magenesium	0.03	0.03	0.04	0.03	0.04	0.04	0.06	0.03	0.08	0.04	0.04	0.04
	Sodium	8.5	8.6	8.9	8.5	8.7	8.6	8.9	8.2	7.9	8.6	8.6	8.5
8	**Texture**	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL	SL

Note:
IS Infected site
DF Disease Free site
CEC Cation Exchange Capacity (m eq. exchange capacity/100g)
SL Sandy Loam

Among different soil parameters analyzed in the laboratory, soil pH seems to play crucial role in determining severity of clubroot disease. The soil in infected areas is found to be acidic in nature, with their pH ranging from 4.5- 5.0. This finding strongly supports the severity of disease in acidic soil as mentioned by other notable researches in the world. (Detailed analysis is made in discussion section), While in disease free sites the pH were measured from 6- 6.5, more towards neutral than the infected sites. There was not so much variation in other parameters of soil as of soil pH, as other parameters were somehow similar in infected sites and disease free site. So, this study re-affirmed the earlier works that acidic soil plays major role to enhance clubroot diseases.

5.2 Socio -Economic findings

5.2.1 Clubroot disease and local perception

In total 75 respondents (25 from each VDC) were administered to local farmers. Among the respondents 53 were female and 22 were male. As more females were involved in farming practices and works, maximum numbers of female (71%) respondents were encountered in the filed and few male respondents were also interviewed. An effort was made to collect local perception and experience form different age groups from 22 to the age of 76.

5.2.2 Knowledge about Clubroot diseases

All the respondents (100%) know about clubroot disease. Locally they called it as Gathey. Gathey means presence of tuber like structures. It shows that every age groups were familiar with this disease incidence. It could be due to that they are facing this disease as threat to their farming practices. Of the total 75 respondents 12 said that cauliflower, cabbage, radish, turnip, mustard and *Rayo* are being infected, 19 respondents noticed only in cauliflower, cabbage and radish, 41 respondents noticed it in cauliflower and cabbage and rest 3 respondents noticed only in cauliflower. This random survey clarified that 16% of the respondents are aware about wide host range of the disease including cauliflower, cabbage, radish, turnip, mustard and *Rayo*. The respondents of this category were mainly from the Sudal and Tathali sites. Twenty-five percentages of the respondents had found clubroot symptom on cauliflower, cabbage and radish plants. Majority of the respondents i.e. 55% from the study area found this disease on the cabbage and cauliflower as the distinct symptoms may well be seen in these crucifers. Very few i.e. 4% respondents saw this disease only in cauliflower. No alternate hosts were noticed by the respondents and it was also not found during the field survey.

5.2.3 Occurrence of clubroot disease in local level

On the experimental sites up to before 5 year no significant disease symptoms were noticed by the farmers in their fields. Among the 75 respondents, 7 % (three from Sudal VDC and two from Tathali VDC) said that Clubroot disease is being observed in the field for the last 5 years. Similarly, 24 noticed it from last 4 years, 33 noticed it from last 3

years and 13 noticed it from last 2 years. Respondents from the Tukucha Nala VDC had noted the disease for the four years only. Then after the frequent emergence of the disease was seen by farmers on all the Studied VDCs. Thirty-two percentage of the respondents had seen the emergence of this disease for last four years that included the 4 respondents from Tukucha Nala VDC, 12 from Sudal VDC and 8 from Tathali VDC. Fourty-three percentage of the respondents that represented 12 respondents from Tukucha Nala VDC, 9 from Sudal VDC and 12 from Tathali VDC was first noted symptoms of the clubroot since 3 year. Seventeen percentages (i.e. Nine from Tukucha Nala VDC, One from Sudal VDC and Three from Tathali VDC) respondents from uneducated background and small scale farming groups had noted the first appearance of this disease for the last 2 years.

5.2.4 Source of seeds

Regarding the source of seeds for this year, 93% respondents bought seed by themselves from local shops at Bhaktapur, Asan and Kalimati seed centers in Kathmandu, while 7% respondents got the seeds from their neighbors.Normally, people with less farm area to cultivate, ask the seeds with the neighbors.

5.2.5 Source of water

As water is crucial for the growth and development of crucifers, 84% respondents were using local canals as the source of irrigation and 16% were using deep well as irrigation source of water.

5.2.6 Loss due to Clubroot disease

According to respondents they do not have exact figure of production loss due to clubroot diseases. The rough estimation in loss of cauliflower and cabbage production was assessed from interaction and survey of the respondents. The rough estimation of the production loss was found 284 kg in the experimental site of Tukucha Nala .The loss increased up to 314 kg in 2065. This indicates 10.56% more loss in 2065 than the year 2064. The same trends of increasing crop losses were observed by the farmers on the other experimental sites too. The estimated loss of 466 kg was found on the Tathali V.D.C during the year 2064 and that of 2065 was found 513. In this site 10.10% more loss than the previous year was estimated. Among the visited sites Sudal V.D.C. hold the

maximum estimated loss of cabbage and cauliflower which was 27.25%. The loss in the year 2064 was approx. 466 kg as equal as Tathali but the loss in year 2065 was significantly high of 593 kg in the study area of the Sudal (Fig: 5.5).

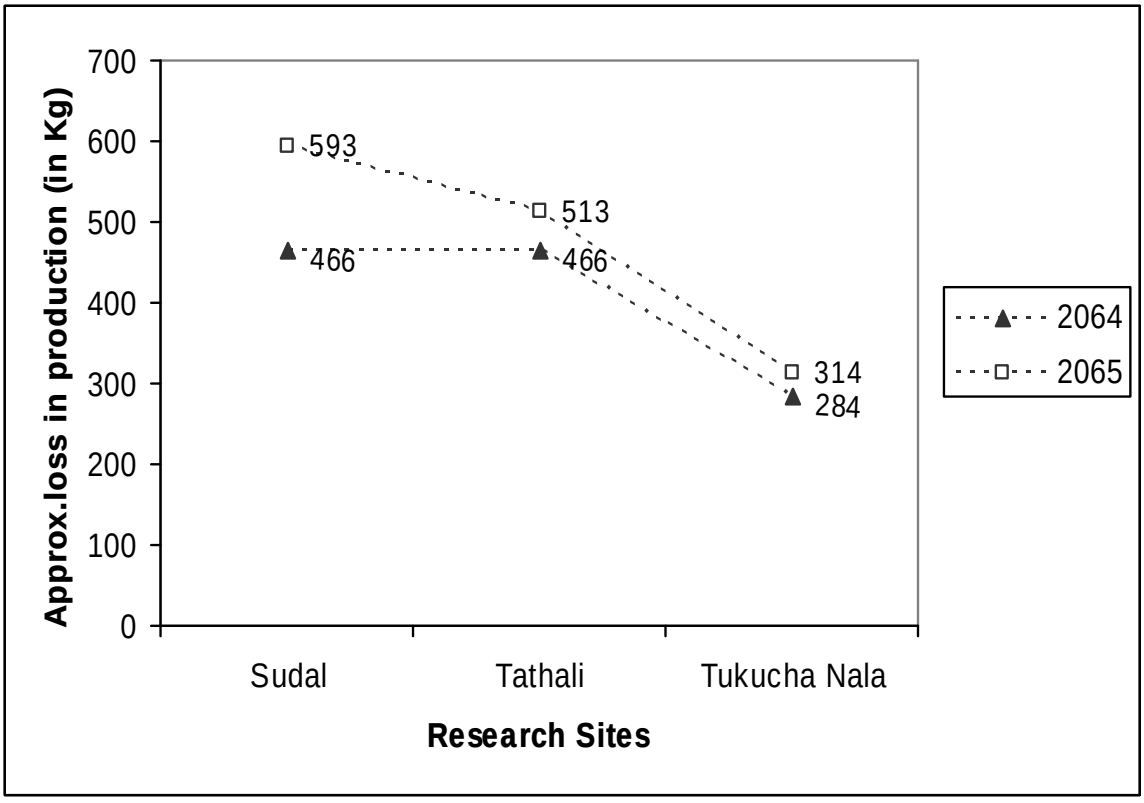


Fig: 5.5. Estimation of production loss due to Clubroot diseases

5.2.7 Transmitting agents of clubroot disease

The transmitting agents play great role in the dispersal of fungal diseases. The survey conducted in the study sites concludes that majority of the respondents had no exact knowledge of the transmission agents .Among the respondents, 3% guessed that the air can be the agent to transmit clubroot disease. Five percentage of the respondents suspect the water may be the cause of the disease transmission .Likewise 13% indicated the dung used in the field may the probable cause of the disease transmission but other 16% blame that fertilizer used in the field may be the cause, as growing use of the chemical fertilizers started on the study area for a few years. In the majority of the disease the seedling also play a crucial role for the disease transmission .In case of this disease too a large bulk of

the respondents of 27% suspected seedlings as a cause of transmission. But the majority among them i.e. 37% had indicated that the soil would be the probable cause of the disease transmission (Fig: 5.6).

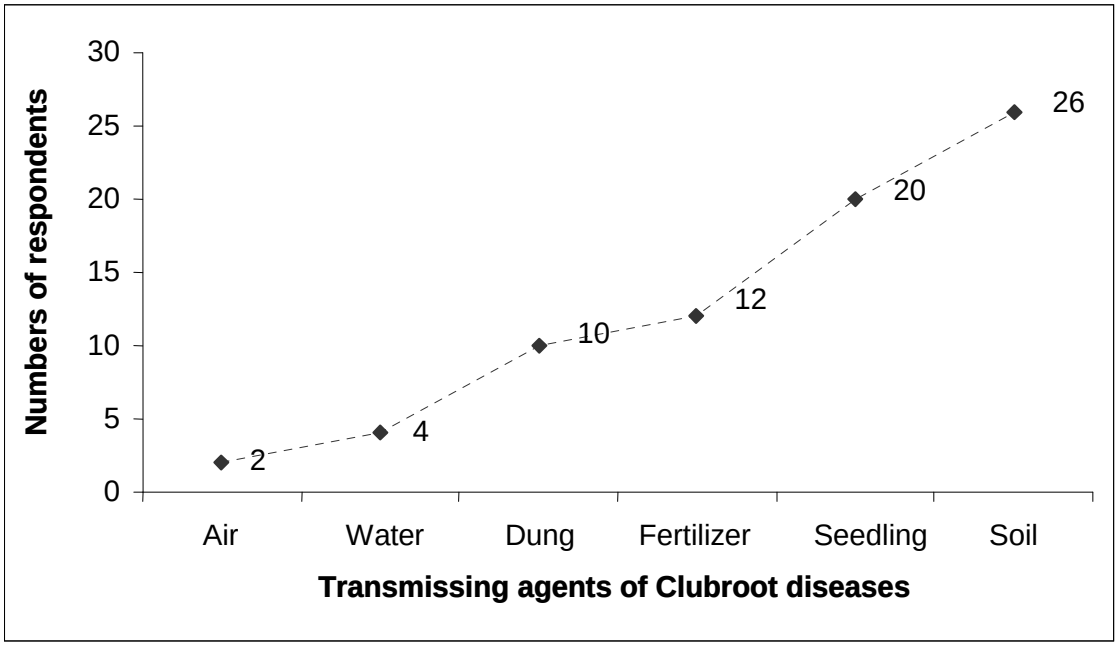


Fig: 5.6. Transmitting agents of Clubroot diseases

5.2.8. Disposal of diseased plants

Compost manure is one of the major sources of the fertilizers for the plants. Majority of the farmers practiced clubroot infected crucifers for making compost manure. Sixty-Seven percentages of the surveyed farmers were found to use the infected plant and plants parts for compost making. Twelve percentages of the respondents used the infected cabbage and cauliflowers to feed cattle. Eleven percentage of respondents buried the infected parts in the soil and 5% respondents used to throw on the road and streets nearby their farm .Burning the plants and plant parts is one of the best method of the disposal of the diseased plants but only five percentage of respondents used to burn the diseased part after they become dried (Fig: 5.7).

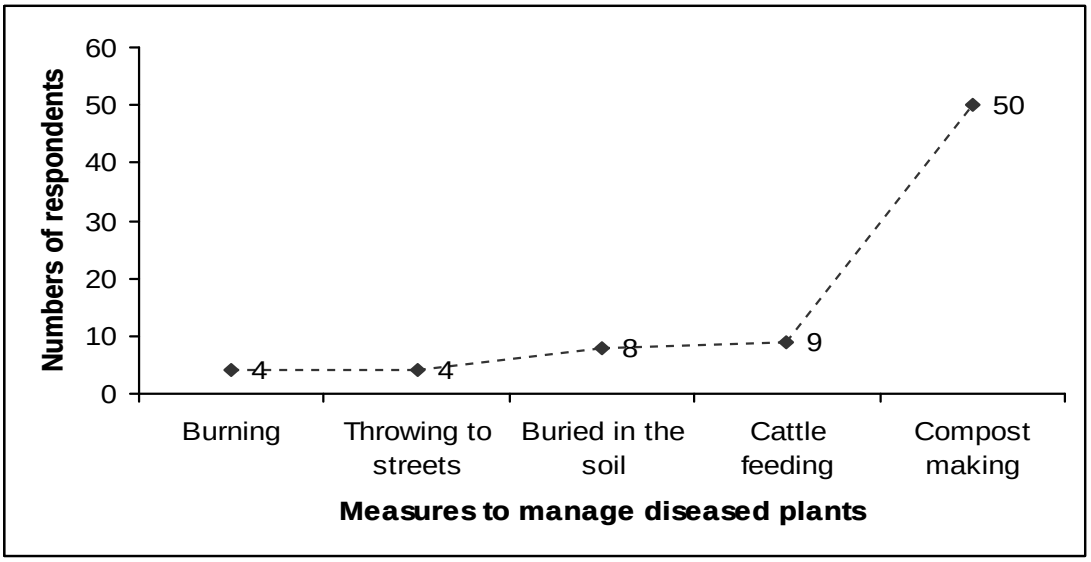


Fig: 5.7. Locally adopting measure to dispose diseased plants

5.2.9. Measures adopting to control clubroot disease

Clubroot disease control is of foremost concern for the profitable production of cabbage and cauliflower. Majority of farms were found to adopt Fungicidal treatment (FT) as the control measures. Among the farmers interacted, 7% left the field unplanted after the emergence of the disease. They left the field to set dry under the sun for 2-3 months thinking that the severity will be minimal for the next cropping. After that they again planted the same crop as these are economically important vegetables. The next bulk of the farmers having the 16% size among the total interviewed respondents had practiced crop rotation with different crops for 2-3 years but they found other crops are not as profitable as compared to these cruciferous crops. Thirty-two percent of the respondents had used lime and fungicides to treat the soil before plantations and used the different fungicides if the disease appeared in the plantlets. The remaining 45% of the respondents said that they practiced the use of the fungicides when the disease appeared in the fields. As they found plants affected from the disease, they uprooted the plants and treated the soil with fungicides. They also practiced treating the plants nearby infected plants, covering the soil with fungicides. (Fig: 5.8).

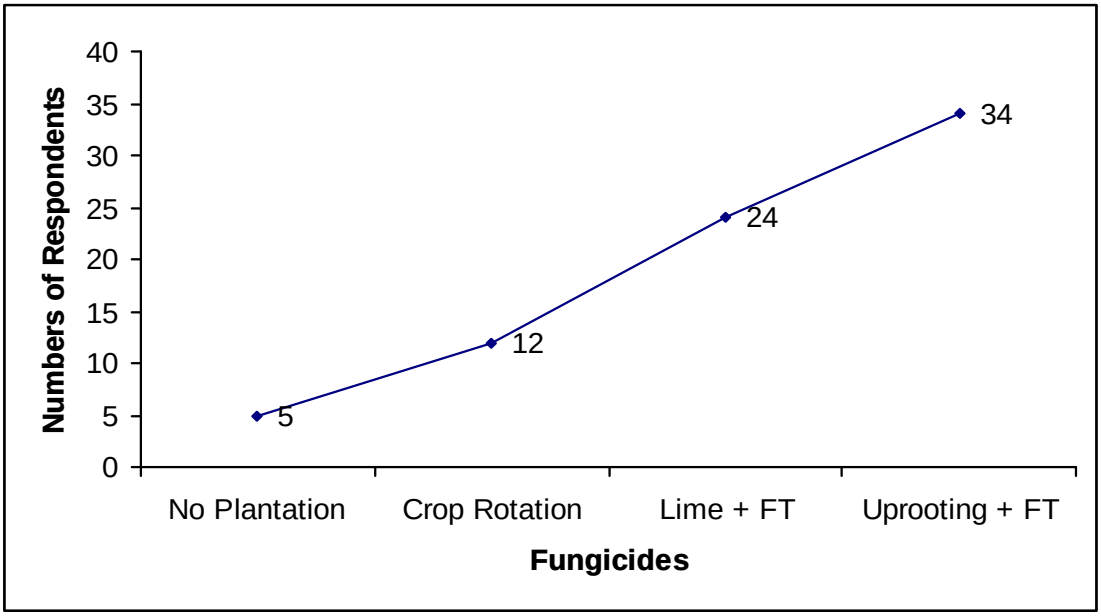


Fig: 5.8. Measures to control clubroot disease

Regarding chemical treatment, local farmers used different fungicides available in the markets. They were using Krilaxyl, Bavistin, Benomyl and Derosal (Fig: 5.9). Majority of growers representing, 35% of the total consulted respondents preferred Bavistin. Other groups of 25% and 27% respondents were found to be using the Derosal and Benomyl respectively. A few (13%) respondents were using Krilaxyl.

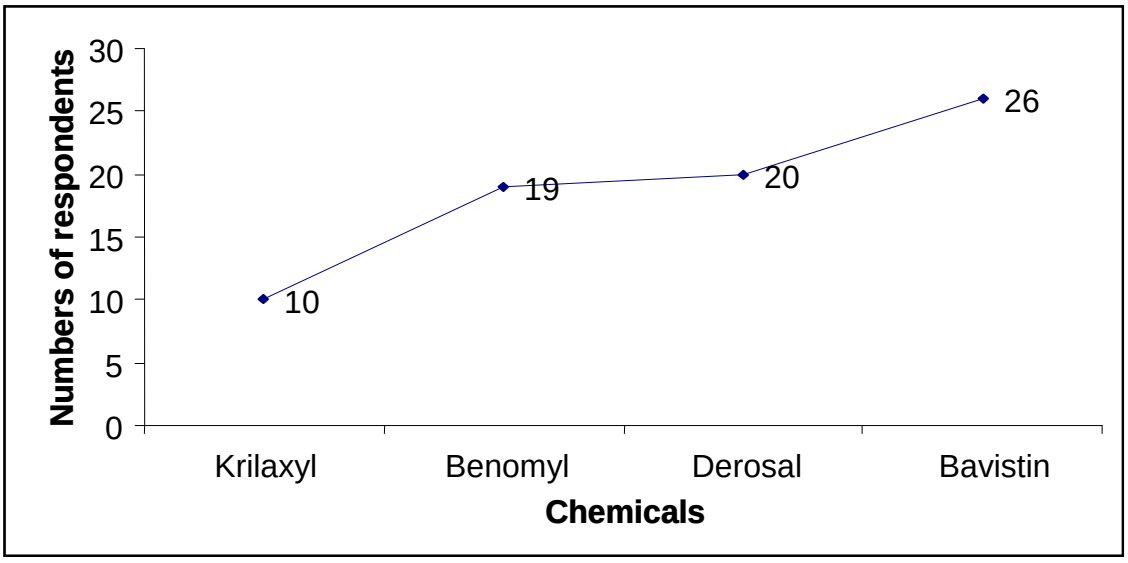


Fig: 5.9. Different fungicides used to control clubroot disease

5.2.10. Home made control measures

Apart from using chemical means to control disease, local people from the research sites were also utilizing their traditional knowledge to minimize the risk and severity of clubroot disease. Of the total, 23 respondents were using mustard cake as a controlling agent and 5 respondents were using extract of *Titepati* (*Artemisia vulgaris)* to control clubroot. While 47 respondents were not adopting such measures (Fig: 5.10).

They used to grind mustard cake with stones and convert to powdery form. Around 1 kg of powder is mixed with 10 liters of water. They used to apply about 100 ml of such mixture in each individual cabbage and cauliflower. This kind of practice is done only once in entire life span of these crucifers. They also used the mustard cake as the fertilizer. Similarly, they were also using extract of *Titepati* (*Artemisia vulgaris)* to minimize the risk of clubroot disease. This practice was normally done in seedling stage. They spray such solution on the leaves of the crucifer and also applied about 50 ml in the root and soil surface of each cabbage and cauliflowers.

The variation in using methods and preparation procedures were seen among the farmers. The traditional cultural practices and the control measures thus used were not so effective control tools. The sites where the home made control measures used were analyzed but no satisfactory results were achieved. Majority of the farmers were found to rely on the modern fungicides.

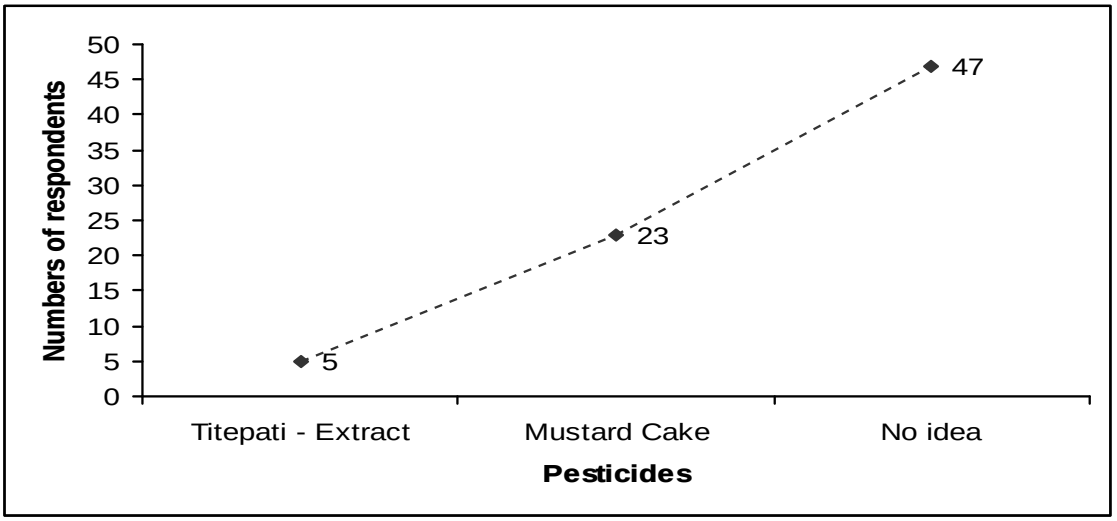


Fig: 5.10. Home made control measures against clubroot disease.

5.2.11. Trainings and Extensions

Trainings and awareness programs play significant role in the management of any diseases. In the research sites majority i.e.73% of respondents (n= 55) complained that they did not get any opportunity to participate in clubroot disease management training programs. But only 20 local people participated in the programs organized by different agencies. Among them, 12 respondents got trainings from District Agriculture Development Office (DADO), 7 got from local level Community based Organizations (CBOs) and only one respondent got training from Nepal Agriculture Research Council (NARC).

Local respondents believed that the control of clubroot is crucial for their healthy agro-products and sustainable livelihood. As majority of respondents depends upon agriculture, they were demanding the attention of concerned authorities to address their problems. Majority of respondents which comprised 43% of the total respondents demanded the urgent need of training and awareness programs for local growers. They believed that the seed which they are getting from the market were not of resistant varieties. Among them 35 % respondents expected from the concerned bodies to have resistant varieties. 23% respondents demanded useful and economic fungicides which can actually control disease in short time period without affecting their yield rate. (Fig: 5.11).

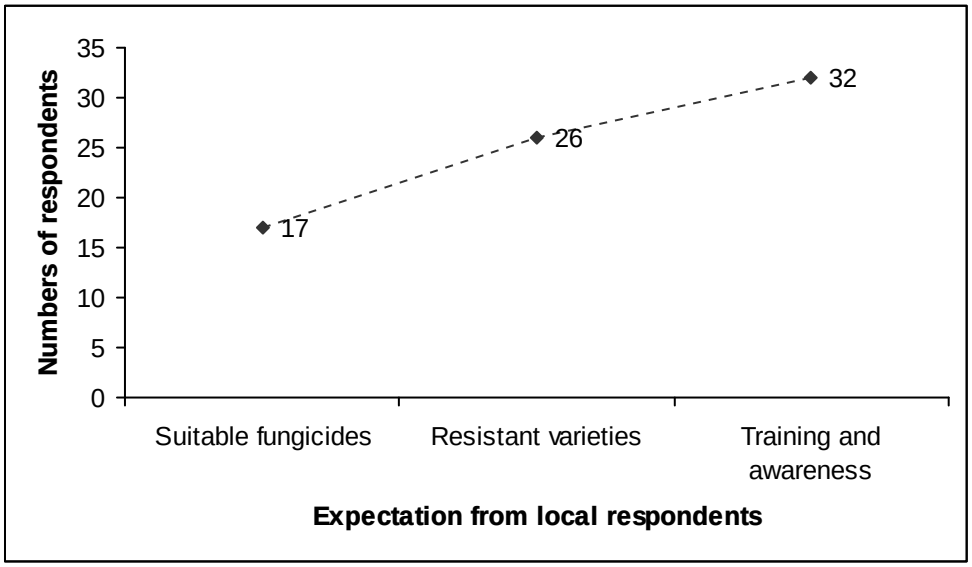


Fig: 5.11.Expectations of local respondents for the control of clubroot disease.

CHAPTER - SIX

DISCUSSION

The present study has been carried out to observe the occurrence of clubroot disease in Tukucha Nala VDC of Kavreplanchok District and Sudal and Tathali VDCs of Bhaktapur District.

Significant yield losses were noted in all three study sites. As the experiment was carried out in the summer season yet the disease intensity was found high. In total 10.38% of the cabbage and cauliflowers was found infected by the clubroot disease. Among the experimented research sites, Sudal VDC had the high disease severity of 10.69% followed by Tathali VDC which had the 10.45%. Likewise in Tukucha Nala VDC, 10.00% of disease occurrence was assessed. Hartman (2008) reported pathogen infestations up to 30-60 per cent of plants causing about 30 to 50 per cent yield loss, while infestations of 10 to 20 per cent lead to 5 to 10 per cent yield loss at Alberta, Canada. Similarly Clubroot occurred up to a maximum of 100% in Chinese cabbage fields in 15 out of 42 locations, and in cabbage fields in 5 out of 13 locations surveyed in several locations in Korea from 1996 to 2000 (PPD, 2003). Typical disease symptoms are widespread, and disease severity has been particularly severe in the Kathmandu Valley and Palung/Daman area of the Makwanpur District. Many cauliflower fields in these areas have had as much as 100% yield loss between 2004 and 2006 with an estimated 40% overall loss from clubroot. The economic loss in this area alone was estimated at $1.4 million in 2004 and 2005 (Timila *et al*., 2008).

In the study area much of the infection occurred in the seedling stages of the crucifers. The transplanted seedlings also show high incidence of disease in the early stage than the later one. In Tukucha Nala, Sudal and Tathali VDC, there was 5.43%, 5.41% and 5.58% respectively of infection on the first visit period which decreased to the 1.21%, 1.20% and 1.81% on respective study area on the last observation period of the field .To maintain the healthy environment farmers should always use plants that have been grown in clubroot free soil or growing medium. Farmers should be aware of movement of seedlings from infested areas to disease free fields (Timila and Shrestha, 2003). The use

of contaminated transplants is the chief means of spread of the causal agent (PPD, 2000). Use of healthy seedlings, non- use of manure from animal fed with infested plant parts, and proper disposal of infected roots are important cultural practices towards control of clubroot disease. (Delhaut and Stevension, 2004).

From the soil analysis it seems that the disease is severe in soil having pH below 6 and in sandy loam soil. The soil of the study area had pH 4.5-5 supporting the acidic nature of the soil. In the disease free area of the experimented plots the soil was found to be of the pH value 6-6.5 which is more towards neutral as compared to that of the disease prone areas. Timila (2006) also reported soil samples from diseased field as sandy loam soils with predominately acidic pH (pH range of 4.2 to 7.2 with >90% below 6.0). The soil types appear to be somewhat non-congenial for survival of the fungus; and in such soils, the persistence of the fungus in the absence of a host may be as short as three years (Delhaut and Stevenson, 2004).The soil of the experimented plot was sandy loam. Observations suggest that poorly drained, sandy soils low in organic matter may be non-compatible, where as well drained, clay type soils are congenial and the fungus will persist for ten years or more (Averre, 2000).

Liming the soil to increase soil pH is also widely practiced in clubroot disease control. In dry soil, liming may fail to suppress because enough moisture in soil is necessary for the movement of alkaline particles to neutralize the acidity around rootlets (Singh, 1985). Disease indices were lower in the plots treated with lime than in the control plots without lime application. The disease index was significantly lower when lime materials were mixed two weeks before sowing compared with four weeks before sowing (Murakami *et al.,* 2002). Liming of the soil in integration with margosa cake amendment was also effective to reduce clubroot incidence of mustard (Chattopadhyay, 1996). The best clubroot control results were obtained with the finest lime materials i.e, hydrated lime, calcitic limestone dust, successful treatments raised pH from 5.7 to 7.0 in the first season and from 6.7 to 7.4 in the second season (Tremblay *et al.,* 2005).

The Clubroot of crucifers was not only confined to cabbage and cauliflower. The present case study found that 19% of the respondents had seen the clubroot symptoms on

cauliflower, cabbage, radish, turnip, mustard and *Rayo*. And 25% of them noticed it on cabbage, cauliflower and radishes. Till date, the disease has appeared in cauliflower, cabbage, broad leaf, mustard, broccoli, radish (rare), mustard and turnip in Nepal (Timila, 2006). In successive seasons, the entire field may become infected. In the visited sites small galls were observed as the study was carried out in the off season cultivation. These affected plants have large galls or clubs on the root and such plants will produce a poor crop or no crop at all (Averre, 2000).

The Study indicates that the disease has been appeared in the study area for the last 5 years and loss due to this disease is increasing year by year. Dasgupta *et al* (1995) noted this disease in India before 7-8 years. In Nepal it was thought to be transmitted from India (Shrestha, 1997). Clubroot had been observed in Nepal since 1993, but severe and widespread epidemics had been observed since 2004 in the Bhaktapur, Kathmandu, Lalitpur, and Palung Valley production areas (Timila *et al*., 2008). It is very difficult to control if once established in a field, because of the soil borne nature of the pathogen and its resting spores are able to survive for more than 18 years even in the absence of Brassica hosts (Toyota and Kimura, 1994; Timila, 2006).

Farmers in the study area are facing larger production losses of cabbage and cauliflower year by year. During the year 2065, 10.56% production loss was estimated in Tukucha Nala research site than that of 2064. Similarly in Tathali 10.10% more loss than previous year was estimated. But the maximum yield loss of 27.25% was estimated in Sudal research site this may due to low pH of soil in that area. Studies made in the past showed increase in club root to yield loss both in biomass and curd weight (Timila, 2006). It has been proved to be a serious problem in Kathmandu and Palung valleys. Up to 100% club root disease was observed on cauliflower in Kathmandu valley (PPD, 2007). Timila *et al*., (2008) had estimated 40% overall yield loss from clubroot in the Bhaktapur, Kathmandu, Lalitpur, and Palung Valley production areas. Scheijgrond and Vos (1954) found a yield reduction ranging from 35-80% depending on the method of calculation on turnip fields by clubroot disease.

Present Study showed that farmers of the study area were not aware about the disposal of diseased plants and parts. Majority of the farmers were using the diseased plant and plant parts for compost making process, which was not the safe way to manage the clubroot disease. But Survival of infectious inoculum of the clubroot pathogen *Plasmodiophora brassicae* was assessed following bench-scale flask composting experiments and large-scale composting procedures (Fayolle *et al*., 2006). Some farmers used these infected plants for cattle feedings. Eleven percentages of the respondents were found to bury the diseased plants under the soil. Some other groups of farmers used to throw the disease plants and plant parts on the nearby roads, streets and other open areas. These methods are not the safe measures. Only 5% of the respondents used to burn the diseased plants although it is the safest measure among the used tools to control the transmission of the disease. Decaying clubs release a large number of newly formed resting spores of *P. brassicae* in the soil, which can remain infectious for periods up to 15 years (Wallenhammar, 1996). Spore germination is expected to be induced by specific substances excreted from host plant roots (Suzuki *et al*., 1992).

There are different transmitting agents of clubroot diseases. Soil containing resting spores may be transported by wind, flood water or more commonly by adhering to farm implements or boots. Farmers of the study area suspected that air, water, dung, fertilizers, seeds and soil could transmit the disease. Suzuki *et al*., (1992) reported that resting spores of the pathogen could survive several years in the soil. Karling (1968) argues that dispersal of *P.brassicae* by rain and water is probably more important than wind dispersal. The disease is not seed borne but, the fungus readily spreads wet surface runoff water, irrigation water, contaminated equipment and to some extent by domestic animals (Sally *et al*., 2000).

Farmers of the study area were adopting different methods to control the clubroot diseases. Some practiced no plantation of the farms for 3-4 months to set the land for soil solarization, especially of the nursery beds. Some other used lime and fungicidal treatment before planting and others practiced uprooting of disease plants and fungicidal treatment of infected areas. Timila *et al*., (2008) had suggested for the soil solarisation of nursery beds in an effort to reduce disease pressure on transplant materials. The effect of

solarisation combined with low rates of soil fumigants on the severity of clubroot and yield of cauliflowers was shown to be dependent on location, on the soil type, water contents and temperature of soil and on the population density of Plasmodiophora brassicae (Porter *et al.*, 1991). The risk of the disease in a field after the absence of a susceptible crop will depend on the levels of the spores (spore load) after the last Brassica crop (Tewari *et al.,* 2005). The disease incidence is more severe, if soils are wet during and after transplanting or seeding (Slocum *et al*., 1990).

Sixteen percentage of the respondents practiced crop rotation on the study area. However the crop rotation was confined to 1-2 years only. As the period of time away from a suitable host increases, the population/concentration of viable spores decreases. Cereals, peas and beans are good break crops (Oxley, 2005). Friberg *et al.,* (2006) had experimented on the efficacy of some non host plants for the management of the *Plasmodiophora brassicae.* Germination of resting spores may be stimulated by certain non host plants. Without a host plant to infect, such germination would lead to a reduced persistence of resting spores in the soil. The effect of four non host plants on *P. brassicae* was investigated in a 3-year field experiment and a 14-month glasshouse experiment. The disease control today is thus restricted to cultural management methods that create an environment less beneficial for disease development, especially avoidance of all host plants, cruciferous crops as well as weeds, in the crop rotation (Hirai, 2006). Reports showed that monocropping of the cauliflower increased the disease incidence from 9% in the first year to 50% in the following year causing 62% yield reduction (Shrestha *et al*., 1995).

Farmers in the study area demanded the effective chemical fungicides to control the disease.Till date no truly resistant cultivars or effective chemical methods are available, cultural practices aimed at reducing pathogen dispersal and propagation are crucial for growers (Young *et al.,* 1991; Höper and Alabouvette, 1996). A number of cultural practices have been followed to control *P. brassicae* infestation including buying or using disease free transplants, using well drained and pathogen free soil, eliminating near by crucifer weeds, incorporating a 7 year rotation with non-cruciferous crops, adjusting soil pH to 7.2 or higher, and using resistant crop varieties (Fallon *et al.,* 1997).

Growers in the study area were also using lime water for the treatment of clubroot disease. They were using lime mixed with the fungicides like Krilaxyl, Bavistin, Benomyl and Derosal. Farmer's experiences and the field study showed that among the fungicide, integration of lime with Bavistin provided the better result. An experiment conducted in the farmer's field at Sipadol, Bhaktapur concluded that application of lime decreases the percent of clubbed plants (PPD, 2007). In field trials conducted over a 5-year period, manipulation of soil pH using lime (calcium oxide) was generally sufficient to prevent significant yield loss on low risk sites. On moderate to high-risk sites, additional protection in the form of calcium and boron and/or protectant fungicides was required to prevent disease (Donald *et al.,* 2006).

Local growers were using Krilaxyl, Bavsitin, Benomyl and Derosal. Majority of cabbage and cauliflower growers preferred Bavistin. Some other preferred Benomyl and Derosal. Only 13% of the farmers preferred Krilaxyl to use as chemical fungicides. Compounds that have given good control of clubroot include: benomyl, captafol (Hoper and Alabavoutte, 1996), chlorothalonil, fostyl-Al, thiabendazole and thiophanate-methyl (Humpherson-Jones, 1993). Fungicides as Fluazinam, Krilaxyl and mancozeb have also been found effective (Dixon *et al*., 1994). Field research in Kathmandu showed the seedling treatment with Benlate (Benomyl) reduced disease incidence and severity of clubroot in cauliflower compared to control (Timila and Shrestha, 2000).

Growers in the study area are adopting different traditional control methods. Farmers of the study area had used mustard cake and *Titepati* as the traditional tools to control the clubroot diseases. But the result was not as satisfactory as the disease severity was not controlled in that areas and their dependency on the chemical fungicides was found dominant. Majority of the farmers used mustard cake as fertilizer too. Timila and Shrestha (2000) had experimented on garlic clove extract to control the disease incidence and severity of the clubroot disease. Liming of the soil in integration with margosa cake amendment was also effective to reduce clubroot incidence of mustard (Chattopadhyay, 1996). The study showed that traditional control of soil borne pathogens has not been successful due to frequent introduction of pathogen through number of ways. In the presence of host, they can multiply rapidly under favorable conditions (Daniel and

Anderson, 1992). Disease management strategies involve the selection and use of appropriate techniques to suppress disease to a tolerable level (Tewari *et al.,* 2005). Different methods have been practiced to control the clubroot disease but once it is established in the field it is hard to control (Timila, 2006).

Local people wish to have some good seeds of resistant varieties. But they are still using the seeds from the local markets. They are seeking the certified seeds for the better crop yield and control of the diseases as they think that resistant seed may help to reduce the disease incidence in the field. Using resistance genotype is one of the easiest and the cheapest method of reducing disease in the field but concerning management of clubroot disease, no host resistance is available (Walker, 1952) and lack of resistance sources was reported by Ludwig-Muller (1999). Despite extensive breeding program for resistant cultivars, the genetic variation in the pathogen has caused problems and very few resistant cultivars have been produced. Some resistant varieties have been produced; the pathogen has overcome the resistance after some time. Resistant varieties exist for radishes, cauliflower (var. Clapton), rutabagas (Swedes) and turnips (Voorrips, 1995).

Twenty-three percentage of the farmers demanded suitable fungicides to control the clubroot disease. For these reasons, no single fungicide has emerged as a truly effective and practical management tool. Pentachloronitrobenzene (PCNB) is currently the recommended fungicide for control of club root (PPD, 2008). PCNB and Benlate (benomyl) have been recommended for control of club root diseases in India (Singh, 1985; Burliegh, 2005). Compounds that have given good control of club root include: benomyl, captafol (Tate and Cheah 1983; Houriuchi and Houri, 1990). Thirty-five percentages of the farmers were expected resistant varieties of cabbage and cauliflower. But there are very limited studies done in Nepal about the resistant varieties. No resistant variety has been identified yet.

Majority of respondents (43%) raised their voice to get opportunity to participate in clubroot disease management training programs and to run clubroot disease management awareness program to the farmers. Awareness is the basic tools to adopt the correct

cultural practices, use of resistant varieties and right and adequate use of the fungicides. Training and the field study programs sharpens farmer's knowledge on plant disease management and plant production. With the view to share experiences on the clubroot disease in Cole crops that has spread in Kathmandu Valley and surroundings causing serious problems in cauliflower and cabbage cultivation and to make strategies against disease management , an interaction program was organized by Plant Pathology Division of Nepal Agricultural Research Council(NARC) on 16 May 2006.Farmers representatives from Kathmandu, Lalitpur, Bhaktapur and Kavreplanchok districts and experts from NARC shared their experiences on disease and disease management measures (NARC Newsletter, 2006).

Very few farmers have got the opportunity to take part in the training program conducted by the different agencies and institutions. Seventy-three percentage of the respondents complained that they haven't got any opportunities of the training programs. A few of the respondents get the opportunity to take the training from different agencies and institution. For this concerned government and non-governmental institutions must pay attention. Nepal Agriculture Research Council (NARC), District Agriculture Development Office (DADO), Tribhuvan University, local level Community based Organizations (CBOs) must conduct effective long term research and awareness programs in the disease prone areas. Millions of Asian rice farmers have been trained in the Integrated Pest Management through "farmer field schools" program. This involves an intensive, hands-on training program following the extension methodology (Witcombe *et al.,* 2006). Rhoades and Booth (1982) argued that involving farmers in the research process increases chance of success in the generation of appropriate agricultural technology. There are a number of well-developed models for farmer participatory research. Participatory plant breeding has been shown to be an effective way to select locally adapted genotypes and to improve farmers' access to useful crop genetic diversity (Sthapit *et al.*, 2006).

CHAPTER - SEVEN

CONCLUSION AND RECOMMENDATIONS

Cultivation of cruciferous vegetables has the highest potential for generating income among more traditional rice and maize farmers in Nepal. Among these vegetables, the most important are cauliflower and cabbage. Although clubroot disease, caused by *Plasmodiophora brassicae* Woronin, has been observed in Nepal since 1993, severe and widespread epidemics have been observed since 2004 in the Bhaktapur, Kathmandu, Lalitpur, and Palung Valley production areas. This disease has been occurring for the 5 years in the present study sites.

Several management practices like lime water treatment, fungicide treatments are being employed to reduce disease severity in these sites. Local farmers were not only using Krilaxyl, Bavistin, Benomyl and Derosal but also adopting extract of *Artemisia vulgaris* and mustard cake.

In order to check the spread of disease and to minimize loss, all the necessary measures should be taken at the local and national levels. Since clubroot infestations are found widely distributed in the study area, various precautionary measures should be taken by the farmers to curb the spread of this disease outside the known infested areas. The following preventive measures would be recommended:

1. Practice good sanitation to restrict the movement of possibly contaminated materials.
2. Use the certified seeds or treated seeds.
3. Avoid the use of straw, hay or green feed, silage and manure from infested or suspicious areas.
4. Awareness and educational programs must be carried out at the farmers' level to disseminate the knowledge of preventive measures.
5. As the disease is soil borne it is very difficult to control it and using long crop rotation practices should be followed for control.
6. Clubroot disease is favoured by acidic soil raising the soil pH could be useful practice to minimize the severity and control the disease.

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Annex I: Questionnaire

Date:
Name: Sex: M /F Age: ……..Address: ……

1. Since last how many years you are growing vegetables? ……
2. <u>List of the vegetable framings:</u>
   Vegetables planted in the field in recent year

Name of the vegetable

3. Have you taken any training relating to vegetable farming? If yes, what type of training and who had provided?

4. From where you have collected the seedlings :
    a. Prepared by oneself
    b. Taken from the neighbors
    c. From nursery or seed shop
5. What is the irrigation source to the vegetable farm?
    a. From channel (Kulo)
    b. From stored water tank (Inar)
    c. Others
6. Do you know about club root disease? If yes what are plants affected by this disease?

7. What are the primary and secondary symptoms of club root diseases?

8. Since last how many years club root disease appears in your field?

9. What to do with the club of the diseased vegetables?
    a. Throw in the field
    b. Throw to the streets
    c. Throw to the water canals or drainage
    d. Burning
    e. Buried in the soil
    f. Making compost

10 From where the club roots diseases is transmitted to your field?

11 In which type of the land /soil the club root disease severity is more?

12 What is the month that the severity of the club root disease is more?

13 What is the month that the infection of the club root disease is severe?

14 What you think that how much estimated loss annually is made by the club root diseases (Income in percentage)

Varieties	Loss in this year	Loss in last year
cabbage		
Cauliflower and like		

15. In your view by what reasons the club root diseases get transmitted?
    a. By seeds
    b. By seedlings
    c. By soils
    d. By irrigation water

e. By air
f. By dung/manure
g. Chemical fertilizer
h. Pesticides/ weapons
i. Others

16. What are the measures you are adopting to control the club root diseases?
    a. Through by uprooting
    b. Use of chemicals or pesticides
    c. Burning of clubs
    d. Shifting of crops
    e. Use of borodeux mixture (*chuna rakhne)*
    f. Others (from where to get?)

17. Have you use any chemicals or pesticides to control the club root diseases ?

Name of pesticides /chemicals	Used time	Using process	Controlling effects

18. Do you ever used home made control measures to control the clubroot disease (Gathe Rog)?

Home made alternatives	Used process	Controlling effects

21. In your Opinion how the gathe rog (Club Root Disease) can be controlled?

22. Any suggestions to concerned authorities like NARC and other institutions?

Thanks