Distribution of Soil Nematodes associated with Grapevine plant in Central Horticultural Centre (CHC), Kirtipur, Kathmandu

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Submitted to

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Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2019

DECLARATION

I here by declare that the work presented in this thesis entitled **"Distribution of Soil Nematodes Associated with Grape Vine Plant in Central Horticultural Centre (CHC), Kirtipur, Kathmandu."** has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of the information have been specifically acknowledged by references to the author (s) or institution (s).

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RECOMMENDATION

This is to recommend that the thesis entitled **"Distribution of Soil Nematodes Associated with Grape Vine Plant in Central Horticultural Centre (CHC), Kirtipur, Kathmandu."** has been carried out by **Ms. Anu Deshar** for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge this thesis work has not been submitted for any other degree in any other institutions.

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On the recommendations of supervisor "Dr**. Mahendra Maharjan**" this thesis submitted by Anu Deshar entitled **"Distribution of Soil Nematodes Associated with Grape Vine Plant in Central Horticultural Centre (CHC), Kirtipur, Kathmandu."** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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This thesis work submitted by **Ms. Anu Deshar** entitled **"Distribution of Soil Nematodes Associated with Grape Vine Plant in Central Horticultural Centre (CHC), Kirtipur, Kathmandu."** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in zoology with special paper Parasitology.

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ABBREVATION

$Cacl_2$	- Calcium Chloride
CHC	- Central Horticulture Centre
masl	- meter above sea level
cm	- centimeter
gm	- gram
µm	- micrometer
mm	-milimeter
GFLV	-Grape vine fan leaf virus

ABSTRACT

Present study was carried out to find out the distribution of soil nematodes in the rhizosphere of grape vine (*Vitis venifera*) in Central Horticultural Centre, Kirtipur. Soil nematodes were extracted from 100 soil samples collected from rhizosphere soil of grape vine i.e. 50 samples each during summer and winter season. Nematodes were extracted by Cobb's (1918) seiving and decantation method and followed by Baermann's (1917) funnel method. The further processing of nematodes was done by Seinhorst's (1959) method in the laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur. Altogether seven species of nematodes were isolated from grape vine rhizosphere soil samples; *Tylenchorhynchus* sp., *Xiphinema* sp., *Longidorus* sp., *Mononchus* sp., *Diplogastrine* sp., *Rhabditis* sp. and *Mesorhabditis* sp. . Among the isolated species, *Tylenchorhynchus* sp., *Xiphinema* sp., and *Longidorus* sp. were parasitic nematodes. *Longidorus* sp. were high in number in summer while *Tylenchorhynchus* sp. showed high infestation during winter season. Beside plant parasitic nematodes, predator nematodes were also isolated from the same soil samples. *Mononchus* sp. was found high in summer season however *Diplogastrine* showed no difference in both summer and winter seasons. Similarly several other free living nematodes were also isolated. *Rhabditis* sp. and *Mesorhabditis* sp. were free living nematodes equally distributed in both summer and winter seasons. In conclusion, the grape vine of Central Horticultural Centre were infected with plant parasitic nematode throughout the year. In order to increase the plant productivity treatment for plant parasitic nematode is necessary and recommended for the same.

1. INTRODUCTION

1.1 General Introduction

Grape vine (*Vitis venifera*) belongs to the order Rhamnales and family Vitaceae. *Vitis venifera* is one of the most ancient precious fruit-bearing plants in the world and played a role of primary importance in civilization for complex societies around the Mediterranean (Mangafa and Kotsaki, 1996). Grapes are grown worldwide on 7.9 millions ha for the production of fresh fruits, juices, raisins, wine and liquors (Jelly *et al.*, 2012). It was spread over Southern Europe and Central Asia during the Neolithic period (McGovern *et al*., 1996). Grapevine domestication has been linked to the discovery of wine (McGovern, 2004). Major grapes are used for winemaking 71% while about 27% are consumed as fresh fruits and only 2% are consumed as dried fruits (Cooper *et al*., 2012). Major grape producing countries are India, Brazil, Venezuela, Peru, Colombia, Thailand, Uruguay, Australia, Mexico, Israel, Egypt (Souza-Leao, 2014). The first commercial vineyard was planted in Western Oregon in 1962 (Pinkerton *et al*., 1999). Grape cultivation was started in Nepal during Rana regime (Dahal *et al*., 2017). Climatic factor, geographical structure as well as soil structure play important role in cultivation of grape vine (Acharya and Yang, 2015). Commercial grape production started in Nepal at Banke and Bardia in 1987 (Atreya and Manandhar, 2016). Grapes are not currently grown in Nepal but with the modern day grafting techniques and the use of terrace landscapes this fruit has been flourished in the hilly region of Nepal (La Mar, 2011).

1.2. Medical Importance:

Consuming grapes play an important role against breast cancer, improve brain function Alzheimer's disease, support eye health and retinal disease, boosts immune system cancer, cataracts prevention properties, helps healing kidney's problems, prevents clots in blood, boost immune power, helps fights viral infection, relieve constipation and good for skin and hair health (Berkowitz, 1996). The fruit juice is mainly used for the treatment of constipation (Lin *et al*., 2003). The bark, wood and root of grape vine are used for the treatment of skin disease (Hasan *et al*., 2009). The aerial part of *Vitis venifera* have been widely used in Ayurveda to treat a variety of common and stress related disorder (Sreemantula *et al*., 2005). The Phytochemicals present in grape such as quercetin, proanthocyanidins, flavonols and phenolic acids have cardio protective properties that reduce mortality by coronary heart disease (Frankel *et al*., 1993): further more they display anticancer properties (Jang *et al*., 1997).

1.3 Disease in Grapevine

Vitis venifera are economically important crop facing many soil borne pathogen and pest that damage or completely destroys a new roots of plants (Aballey *et al*., 2009). Crown gall disease has been recognized as a plant disease of worldwide importance on many

plant species (Burr and Otten, 1999). It may also cause significant losses in nurseries by affecting vine growth and making the destruction of infected plants (Cavara, 1985). In addition leaf roll disease is also viral disease of grape vine that occurs in all of world's grape growing area which causes significantly yield losses, delayed fruit ripening and overall decline in vine vigor (Martelli and Boundon –Padieu, 2006). Likewise Black foot is a grape vine trunk disease that affects young grape vine which shows symptoms like sunken necrotic root lesions with reduction in root biomass and root hairs. Another disease that affect grapevine is Red Blotch which was discovered in Northern California in 2008 and has become a major headache for wine industry in USA (Sudarshana *et al*., 2015).

1.4 Nematodes in grape vine

Globally, nematodes are serious threats in vineyards (Anwar and Van Gunday, 1989). The most serious direct damage is caused by *Meloidogyne* sp., *Xiphinema* (Brown *et al*., 1993) while nematodes like *Criconemoides* sp., *Paratylenchus* sp., *Helicotylenchus* sp., *Rotylenchus* sp., *Longidorus* sp., *Paralongidorus* sp. and *Trichodorus* sp. are less likely to cause serious damage in the grape vine (Boubals and Dalmasso, 1964). Among several parasitic nematodes affecting grapevine *Meloidogyne* sp., *Paratylenchus* sp., *Pratylenchus* sp. as well as *Xiphinema* sp. have been reported from America in the state of Oregon (Pinkerton *et al*., 1999) and in Washington by Zasada *et al*., (2012). On the other hand, One of the most serious disease problems affecting grape vine production is the grape vine fan leaf virus (GLFV) transmitted by *Xiphinema index* and *X. italiae* (Hewitt *et al*., 1958). Moreover, *Mesocriconema xenoplax* was the most common plant parasitic nematodes found in vineyards in Spain by Pinochet and Cisneros, (1986) in Germany by Weischer, (1961), in France by Scotto La Massese *et al*., (1973) and in Switzerland by Guntzel *et al*., (1987).

1.5 Common plant parasitic nematodes

A)*Tylenchorhynchus* sp.

Classification

Class-Secernentea

Subclass-Diplogasteria

Order-Tylenchida

Sub order-Tylenchina

Super family-Tylenchoidea

Family-Belonolaimidae

Sub family-Telotylenchinae

Genus-*Tylenchorhynchus*, Cobb, 1913

Tylenchorhynchus is soil dwelling stunt nematodes with about 8% of total known species as parasities (Anderson and potter, 1991). According to the Fortuner and Luc, 1987 the general characteristics of *Tylenchorhynchus* are medium sized body, prominent phasmids and cephalic frame work slight to heavy sclerotized. Male have a wide range in annule size and a different pattern of body annulation than that of female (Anderson, 1983). *Tylenchorhynchus* mainly parasitise graminae family such as rice, wheat, maize (Upreti, 2000) which has been reported from both upland and low lands of Nepal (Pokharel and Regmi, 2000). These nematodes had been reported on tobacco root (McIntyre and Miller, 1978) in vineyard of India (Bhatia and Gupta, 1973) and in Australia (Striling, 1976). *Tylenchorhynchus mashhoodi* has been reported from vegetable crops of Kathmandu valley (Keshari and Gupta, 2016) and also from pear (Shrestha, 2015).

B)*Xiphinema* sp.

Classification

Class-Enoplea

Subclass-Dorylaimia

Order-Dorylaimida

Sub order-Dorylaimina

Super family-Dorylaimoidea

Family-Longidoridae

Subfamily-Xiphinematidea

Genus-*Xiphinema*, Cobb, 1913

Xiphinema are large nematodes with an adult size 1.5 to 5 mm (Whitehead, 1998). They have long protrusible odontostylet with 3 basal flanges at the posterior end of stylet (Evans et al., 1998). *Xiphinema* was reported from grape vine (Pinkerton *et al*., 1999; Anonjou, 1981) vegetable (Bhatta, 1967) maize (Sharma–Poudyal, 2004) *Pinus*, *Alnus* and *Quercus* trees (Sharma, 2003) apple (Sudershan *et al*., 2002).

C)*Longidorus* sp.

Classification

Class-Enoplea

Sub class-Dorylaimia

Order-Dorylaimida

Sub order-Dorylaimina

Super family-Dorylaimoidea

Family-Longidoridae

Genus-*Longidorus*, Cobb, 1913

Longidorus sp. are large plant nematodes whose size range from 1.5 to 12 mm in length in the adult stage (Hunt, 1993). The inner layer of cuticle usually widens at the neck and tail end and has a very faint transverse striation that has a radical appearance on tail (Southey, 1978). Female of *Longidorus* have two ovaries that are opposed and reflexed (Ye and Robbins, 2004). *Longidorus* sp. have been reported from cauliflower, chilly, wheat, paddy, cabbage and tomato (Amatya and Shrestha, 1996) and from pear (Shrestha, 2015).

1.2 Objectives

1.2.1 General objective

To find out distribution of soil nematodes associated with Grape vine plant in Central Horticultural Centre (CHC), Kirtipur, Kathmandu.

1.2.2 Specific objectives

i) To study taxonomy of plant parasitic nematodes of grapevines.

ii) To determine distribution and density of plant parasitic nematodes around rhizosphere of grape vine in CHC, Kirtipur.

ii) To determine distribution and density of predatory and free living nematodes

1.3 Rationale of study

Present study was carried out at Central Horticulture Centre, Kirtipur where grapes (*Vitis venifera*) occupies considerable production in fruit production. As we already know, there are many migratory nematodes including tomatoes, potatoes, grapes and many other plant which cause injury including *Tylenchorhynchus* sp., *Longidorus* sp. and *Xiphinema* sp.. Furthermore, there are many nematodes that parasitize to the grape plants. Parasitic nematodes attack different parts of plants, lower significantly the production of plant if infected heavily and this is limiting factor in agricultural production. It is necessary to identify nematode pests and to understand their biology.

The research on plant nematodes and their identification in Nepal is very few. This information may be useful to control nematodes in grape vine and consequently increase production. Present study was then focused to analyse the plant parasitic nematodes associated with grape vine.

2. LITERATURE REVIEW

The first described plant parasitic nematodes were discovered in wheat seeds (*Anguina tritici*) by Needhams in 1743 (Thorne, 1949). Plant parasitic nematodes are recognized as major agricultural pathogens which causes huge lost in agriculture in worldwide (Sasser and Freckman, 1987). Out of Known nematodes species 50% are free living which inhabits in soil or fresh water, 25% are marine found on sea water, 15% are animal parasites and 10% are known as plant parasitic nematodes (Pokharel and Larsen, 2007). About 40% of free living nematodes rely on bacteria fungi and protozoan, whereas about 45% of other nematodes feed on animals while other remaining 15% depends on plants (Lambert and Bekal, 2002). Among the plant parasitic nematodes, a couple of nematodes animal varieties feed on aeronautical plant parts (leaves, stems, flowers and seed), but most of nematodes feed underground plant parts (roots, bulb and tubers) (Pokharel and Larsen, 2007). All plant parasitic nematodes have stylets: a solid, empty, needle like structure that is utilized to penetrate a plant cells, infuse nematode discharge and to benefit from plant cell contents, stylets fluctuate fit as a fiddle and size as indicated by the sustaining system of nematodes: for instance, nematodes, for example, *Trichodorus* that feed on epidermal cells have short stylets where as those, for example, *Xiphinema* sp. or *Longidorous* sp. have longer stylets and can benefit from cells more profound with in the plant (Gheysen and Jones, 2006).

It has been claimed by Robbins, (1993) that six species of *Xiphinema* namely; *X. diffusum, X. floridae, X. laevistriatum, X. luci, X. shell*, and *X. tarjanense* were found in Florida, while *Mesocriconema xenoplax*, *Xiphinema americanum*, *Pratylenchus* sp. *Meloidogyne halpa* have been reported from vineyards of Oregon (Pinkerton *et al*., 1999). Similarly, other plant parasitic nematodes like *Pratylenchus vulnus* and *Paratylenchus hamatus* were discovered in California (Ferris and Mckenry, 1975) *Xiphinema* sp. and *Paratylenchus* sp. were also reported from Australian continent (Quader *et al*., 2003). On the other hand, plant parasitic nematodes have been reported from European countries also; such as *Longidorus elongatus*, *L. orongorongensis* , *Paratrichodorus lobatus* from New Zealand (Sturhan *et al*., 1997) *Xiphinema vuittenezia, X. Pachtaicum, Paralongidorous maximus* from Austria (Gangl *et al*., 2009).

Several species of plant parasitic nematodes associated with varieties of plants have been recorded from China i.e *Pratylenchus teres, Hoplolaimus pararobustus, Criconemoides complexus* and *Hemicriconemoides* sp (Liu and Feng, 1995). Similarly, several species of plant parasitic nematodes around banana plant has been extracted by Khan and Hassan (2011) like *Pratylenchus coffeae*, *P. brachyurus, P. similis*, *Meloidogyne incognita*, *M. Javanica, Hoplolaimus indicus, Rotylenchulus reniformis*, *Helicotylenchus multicinctus, H. abunaamai, H. incisus, H. gratus, H. dihystera*, *Tylenchorhynchus nudus, T. mashhoodi, T. coffeae*, *Hirschmanniella mucronata* and *Criconemoides* sp. from India and indicated that among them *P. coffeae, P. brachyurus*, *M. incognita*, *H. multicinctus* and *R. reniformis* infect highly in banana plant. From India itself five species of plant parasitic nematode also have been recorded from coconut plant (Khan *et.al*., 1971), i.e

Dolichorous pulvirus, *Discocriconeemalla recensi*, *Longidorus sagirus*, *Paralongidorus flexus*.

Eighty five plant parasitic nematodes belonging to 38 genera i.e *Meloidogyne incognita*, *M. arenaria*, *Heterodera avenae*, *Aphelenchoides composticola*, *Ditylenchus myceliophagus*, *Rotylenchulus reniformis*, *Pratylenchus coffeae* and *Tylenchulus semipenetrans* were recorded in vegetable from Punjab (Mahajan and Kaur, 1991; Mahajan and Chhabra, 2009). *Diplenchus indicus* was recorded from rhizosphere of grape vine from India (Khan *et al*., 1969).

In Nepal, root knot nematode (*Meloidogyne* sp.), *Hirshchmanniella oryzae*, *Aphelenchoides besseyi*, *Ditylenchus angustus*, *Tylenchorhynchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp. were widely distributed in rice (*Oryza sativa*) (Pokharel and Regmi, 2000; Pokharel, 2001 and Sharma Poudyal *et al*., 2002). Upreti (2000) reported *Meloidogyne raminicola* is more prominent in light soil than in heavy soils. Species of *Tylenchorhynchus*, *Hemicycliophora*, *Pratylenchus*, *Helicotylenchus Hoplolaimus Rotylenchus* ,*Cricinemoides* and *Xiphinema* were associated with rice and other species of plant parasitic nematodes such as *Anguina*, *Aphelenchus*, *Pratylenchus*, *Heterodera*, *Xiphinema* and *Hoplolaimus* were associated with wheat (Upreti, 2000). Amatya and Shrestha (1969) reported *Helicotylenchus* sp., *Pratylenchus* sp., *Heterodera* sp.,*Meloidogyne* sp., *Anguina* sp. and *Tylenchorhynchus* sp. from vegetables. *Tylenchorhynchus mashhoodi*, *Hoplolaimus indicus*, *Helicotylenchus incisus*, *Microposthonia paraxestis* and *Hemicriconemoides cocophilus* were recorded as plant parasitic nematodes from vegetable in hilly district of Kathmandu (Keshari and Gupta ,2016). Sharma *et al*., (2001) reported a new species of *Cactodera johanseni* for the first time from raddish of Bhaktapur. *Pratylenchus* sp., *Meloidogyne* sp., *Tylenchorhynchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *Rotylenchus* sp., *Xiphinema* sp., *Trichodorus* sp., *Hirshmanniella* sp. and *Longidorus* sp. were recorded from papaya and maize (Pokharel *et al*., 1994; Sharma –Poudyal, 2004). A total of 32 species of nematodes were reported to be plant parasitic in Nepal (Gupta, 1997).

Stunts nematodes *Tylenchorhynchus* sp. are economically important plant pathogen which affects different plant species of world. Striling, (1976) reported *Tylenchorhynchus* sp. from grape vine in Australia. Two species of plant parasitic nematodes i.e *Tylenchorhynchus quaidi* and *T. tritici* were recorded from potato of Pakistan (Golden *et al*., 1987).

The first record of *Tylenchorhynchus* sp. in India was published in an abstract of paper Siddiqi and Basir, (1959). Saxena *et al*., (1977) observed that the application of mustard oil cake (500kg/ha) was effective in controlling *Tylenchorhynchus* sp. on grape vine. *Tylenchorhynchus brassicae* plant infects cabbage and cauliflower vegetables (Alam and Khan, 1974). On experiment it has been reported that the population of these nematodes were associated with the fertilizers that farmers use frequently. It was found that the population of *T. brassica* was reduced around the root of cabbage and cauliflower where oil cakes were used as fertilizers than inorganic fertilizers (Alam and Khan, 1974;

Siddiqui *et al*., 1976). Zaki and Mantoo, (2003) reported *T. nudus* from different fruit (cherry, walnut, apple, apricot, plum and pear) of Kashmir valley. Dabur (2000) reported *T. mashhoodi* were found prevalent where rice plantation is frequently practiced.

T. mashhoodi, *T .annulatus* have been reported from vegetable crops of Kathmandu valley (Keshari and Gupta, 2016; Pudasaini and Bert, 2000). Yadav *et al*., (1989) and Pokharel *et al*., (1994) have reported *Tylenchorhynchus* sp. from Papaya and also from Pear (Shrestha, 2015).

Various species of *Longidorus* i.e *L. attenuatus*, *L. elongates*, *L. intermedius*, *L. juvenilis*, *L. macrosoma* and *L. posseneckensis* were isolated from rhizosphere of grape vine *Vitis venifera* in Austria (Tiefenbrunner and Tiefenbrunner, 2004). Similarly, other *Longidorus* species such as *L. africanus*, *L. crataegi* from grape vine of Portugal (Antonia *et al*., 1995) *L. apuloides* from Italy (Roca and Francesco, 1996), *L. carpatnius*, *L. piceicola* and *L. guglansicola* in Russia (Liskova *et al*., 1997), *L. jagerae*, *L. attenuatus*, *L. elongatus*, *L. enonymus* and *L. leptocephalus* from carrot and sugarcane in Africa (Heyns *et al*., 1998; Spaulla *et al*., 1991), *L. israelensis* in carrot from Israel (Pereva *et al*., 1998).

Longidorus siddiqii has the distribution of 13.8% in banana (Kaur *et al*., 2010). *Longidorus africanus* causes 5% loss in vegetable crops in Punjab, India (Anwar and Mckenry, 2012). *Longidorus dimorphicaudatus* and *Longidorus mirus* from maize in India was recorded by Baniyamuddin and Ahmad, (2006).

Amatya and Shrestha, (1996) have recorded *Longidorus* sp. from cauliflower, chilly, wheat, paddy, cabbage and tomato and Shrestha, (2015) in pear tree in summer and autumn sample in Nepal.

Sasser, (1989) reported the various species of *Xiphinema* from different economically important plants such as Banana, Grapes, Sugarcane, Citrus from different countries of world. About 37% of vineyard has been affected by *Xiphinema americanum* (Pinkerton *et al*., 1999).

From the research of Roca *et al*., (1992) revealed that *X. belmontense* and *X. abrarticum* were found in apple and peach in Portugal. Taylor *et al*., 1994 studied on distribution of *X. diversicaudatum* in an uncultivated woodland habitat in England and cultivated soil in Scotland .They found out that the nematodes have distribution in both cultivated soil and uncultivated soil. *X. pachtaicum*, *X. index*, *X. italiae*, *X. inges*, *X. vuittenezi*, *X. diversicaudatum* were found to be infested in grape vine in Cyprus (Anonjou, 1981) and in Iraq (Stephan *et al* 1985).

Feil *et al*., (1997) studied the 'effect of seasonal and site factor on *Xiphinema index'* population in two vineyard of California which resulted the greater number of nematodes occurred in Winter month. Also the research had concluded that the extremely high and low soil temperature corresponded to low nematode number.

In Austrila Stirling, (1976) reported *Xiphinema* sp. from vineyard. Taylor and Brown , (1997) stated that 4% of *Xiphinema* have been shown to transmit certain nepovirus to wide range of fruit and vegetable crops whereas Shakeel *et al*., (2012) had accounted that *Xiphinema* sp. causes 5.1% of loss in vegetable crops in Pakistan. In Like manner Robbins *et al*., 2000 observed *Xiphinema* sp. from rhizosphere of Yulan plants in China

Xiphinema americanum cause 7% loss in vegetable crops in Punjab, India (Anwar and Mckenry, 2012). Bhatta, (1967) reported plant parasitic nematodes i.e *X. index* and *X. diversicaudatum* from Kathmandu, Nepal. Sharma –poudyal, (2004) recorded *Xiphinema* sp. from maize of Chitwan. Sharma, (2003) have recorded *Xiphinema* sp. from the rhizosphere of Citrus, *Pinus,Alnus,Quercus* trees from different localities of Kathmandu. Sudershan *et al*., (2002) reported *Xiphinema mali* from apple in Nepal.

The history of *Mononchus* goes back to 1845 when Dujardin described *Oncholaimus muscorrum*, *O. fovearum* and *Enoplus crassiculus.* However Bastian, (1865) proposed the type genus *Mononchus* and described five new species i.e *M. truncatus*, *M. papillatus*, *M. macrostoma*, *M. tunbridgenesis* and *M. cristatus*.

Clark, (1960) described *Mononchus* from New Zealand, Cananda Mulvey, (1961), Singapore Ahmad *et al*., (2005). Coetzee, (1965) described several known and new species of *Mononchus* from South Africa, Malaysia (Loof, 2006). Khan and Araki, (2002) described a few species from Japan. Jairajpuri, (1971) reported many genera of *Mononchus* from India while Badri *et al*., (1978) described *Iotonchus* and new species of *Cobbonchus* sp. from India. Zaki and Mantoo (2003) have reported the occurrence of one predaceous species of *Mononchus* sp. in the fruit trees in Kashmir valley.

In the context of Nepal, *Mononchus aquaticus* was reported from major vegetable crops from Bhaktapur and Kavre districts of Nepal by Keshari *et al*., (2018). Chettri and Subedi , (2019) reported *Mononchus* from kiwi plant likewise Shrestha, (2015) founded in Pear Plants.

Chitwood, (1951) reported marine nematode from North America while in Mexico it was founded by Chitwood and Timm, (1954). Loof, (1964) reported free living nematodes i.e *Mesorhabditis capitata* and *M. szunyoghyi* from Venezeula. Chinnadurai and Fernando, (2006) reported 6 species of free living nematodes i.e *Ptycholaimellus ponticus, Paracomesoma dubium, Desmodora tenuispiculum, Camacolaimus barbatus, Haliplectus dorsalis* and *Thalassomon hystera* and 1 genus *Pseudolella* sp of free living belonging to the 2 order and 7 families for first time in India. Likewise Krishnamurthy *et al*., (1984) recorded 27 genera and 4 species of free living in India.

Kanazaki *et al*., (2012) have reported *Pristionchus fissidentatus* free living from the soil in Nepal. *Rhabditis* and *Mesorhabditis* were recorded from kiwi plants by Chhetri, (2017) and also from Pear by Shrestha, (2015).

3. MATERIALS AND METHODS

3.1 Study Area

The present study was carried out at Central Horticulture Centre Kirtipur. This study area is located next to Bhajangal in Bagmati Zone, Central Region, Nepal. It has a length of 1.85 km. Geographically it lies in between 27˚40'E latitude and 85˚17' N longitude, at an altitude of 1320 meter above sea level (CHC, 2016). It is surrounded by the Bagmati River in the east, Chandragiri municipality in the western north and Dakshinkali municipality in the south. The total area covered by CHC is 20 hectares, of which 11 hectares is occupied by 21 different fruit orchards, including *Vitis venifera* (CHC, 2016).

Photo 1: Map of Central Horticultural centre

3.2 Study Period

The sample were collected in two season from Central Horticulture Centre. First sample was collected in summer from May to June 2018. Second sample was collected in winter from Dec 2018 to Jan 2019.

3.3 Sampling

The present study was conducted to record the different parasitic nematodes and non-parasitic nematodes from the rhizospheres soil of grape vines from CHC. Soil samples were collected from the rhizosphere of 50 mature grape vines selected randomly at the study site. The soil sample was collected from rhizosphere of selected plants about 2 feet

away from main stem of grape vines and about 30 cm below the surface of the soil. The soil was dug by iron rod and hole was made. From this hole in about 30 cm depth, about 500 gm of soil was collected in a polythene bag. The sample was labeled by the marker, sealed and kept in cool place. The samples were then brought to the laboratory of Central Department of Zoology, T.U. Kirtipur for further processing and identification of the soil nematodes.

Photo 2: Collection of soil samples

3.4 Physical Requirements

The materials needed for this study includes:

i) Digging rod Funnel
ii) Supporting nets
iii) Tissue papers
iv) Wooden stands
v) Rubber pipe
vi) Clip
vii) Needle
viii) Cavity block
ix) Desiccator
x) Slide/Cover Slip
xi) Binocular microscope
xii) Compound microscope
xiii) Ocular micrometer
xiv) Hot plate
xv) Nail polish
xvi) Measuring tape
xvii) Polythene bags
xviii) Marker

xix) Weighing balance
xx) Sieve
xxi) Bucket
xxii) Dropper

3.5 Chemical Required

i) Formalin
ii) Triethanolamide
iii) Distilled water
iv) Ethanol
v) Glycerine
vi) Calcium chloride
vii) Grease
viii) Purified wax

3.6 Preparation of chemicals

For the processing and fixation of collected samples of nematodes mainly two solution were prepared in laboratory. The chemicals used were TAF solution for fixation and Glycerol Ethanol solution for processing.

3.6.1 TAF solution

TAF solution works as fixative which is used to fix the nematodes. The appearance of nematodes after fixation in TAF is remarkably life like. The solution remains stable for the long time. Triethanolamide neutralizes any free formic acid and being hygroscopic. It prevents specimens from drying even if the fixative evaporates. TAF solution was prepared by mixing following chemicals in the given proportion.

7ml- Formalin (40% formaldehyde)

2ml-Triethanolamide

91ml- Distilled water

3.6.2 Glycerol-alcohol

Processing nematodes by Seinhorst's glycerol ethanol method gives very good results. It is very quick and labour saving. This solution is prepared by mixing the following chemicals in the given proportion

95ml-30% ethanol

5ml - Glycerol

3.7 Processing of soil samples

The collected soil samplewere processed by Cobb's (1918) modified sieving and decantation technique followed by Baermann funnel technique (Baermann, 1917). In this method, about 500 gm of soil sample was taken in the bucket and half filled with water. The muddy suspension was gently stirred with hand and lumps were broken down. Stones

and debris were removed. The muddy mixture were stirred and poured through sieve of mesh size of 500 µm, 300 µm and 150 µm respectively. The residue was discarded and filtrate was collected in another bucket. It was passed into another bucket through the sieve of mesh size of 75µm. The suspension was washed by gentle flow of water and filtrate was discarded. Residue in the sieve was collected with clean water in beaker. This residue was poured over the tissue paper mounted on the course of supporting net, place in Baermann funnel. The stem of funnel was connected with rubber tube and closed it's tip by clip. The funnel was filled with water to the level just touching the lower surface of tissue paper on supporting net, avoid bubbles. Apparatus was left undisturbed at least for 24 hours. A small quantity of suspension was later collected through the tip of rubber tube in a cavity block. Nematodes move toward the bottom by penetrating towards the tissue paper so that they could be collected from the few drops of water at the rubber tube in the petridisk.

Photo 3: Processing of soil sample by Seiving method

Photo 4: Baermann funnel method of soil nematodes extraction.

3.8 Killing and fixation of nematodes

The nematodes suspension collected in the petri dish was left undisturbed for few minutes allowing the nematodes to settle down. Excess water was drawn out using pipette. The collected nematode was transferred in the cavity block and hot TAF solution was added in it. This hot TAF solution kills nematodes as well as acts as fixing material. It prevents the destruction of epidermal layer of specimens and preserves the nematodes in the appearance of remarkably life like.

3.9 Dehydration of nematodes

For the preparation of permanent mounts, nematodes were transferred to a cavity block. Then again transferred into another cavity block containing glycerol ethanol solution by picking the nematode with the help of fine needle. These cavity blocks were kept in desiccator containing anhydrous calcium chloride for about 2 weeks at room temperature for slow dehydration.

Photo 5: Dehydration of Nematodes in dessicator.

3.10 Mounting and sealing

The dehydrated nematodes were ready for mounting. A drop of anhydrous glycerin was placed on slide and the nematodes were transferred from cavity block to this drop by using the wax ring technique for permanent mounting. For extra support for slide, water coloured nail polish was also applied around the coverslip.

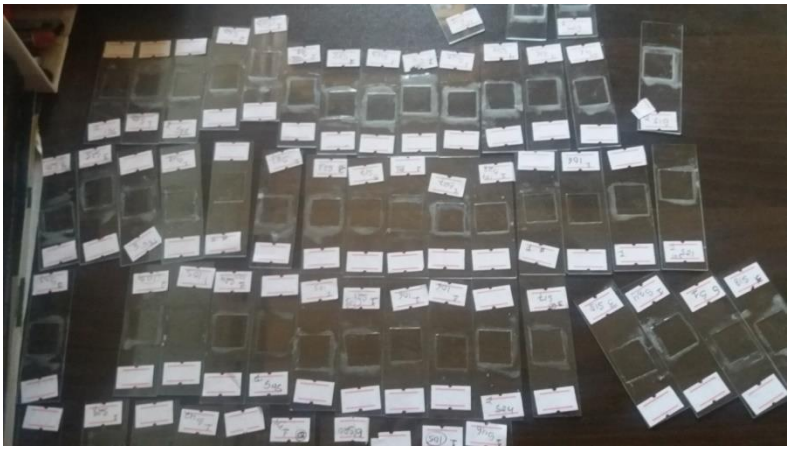

Photo 6: Preparation of Slides

3.11 Observation and identification

The slides were observed under the low power and high power magnification i.e 4X, 10X and 40X for nose piece and 10X for eye piece. The characters were then compared with the taxonomic keys of Smart and Nguyen (1998).

Photo 7: Observing under microscope

3.12 Morphometric analysis for species identification

Morphometric analysis of *Xiphinema, Longidorous, Tylenchorhynchus* was carried out to determine the species. In this process the detail structure and parts of body was measured with the help of ocular micrometer

3.13 Callibration of microscope

The microscope was calibrated with stage and ocular micrometer and following calibration factor was calculated for each objective.

Eye piece	Objective	Calibration factor
10X	4X	31.6
10X	10X	9.9
10X	40X	2.4

4. RESULT

Health of plant directly influences the productivity of the plant. Grape vine plants have been reported to be infected by various disease including plant parasitic nematodes .To analyse the plant parasitic nematodes of Grape vine, soil samples from rhizosphere were screened. The result revealed the presence of seven different species of nematodes .The isolated nematodes were classified into three groups; plant parasitic, predator and free living on the basis of morphological characters. Taxonomy was carried out to confirm upto the genus of the parasitic nematodes which includes *Tylenchorhynchus* sp., *Xiphinema* sp. and *Longidorus* sp. with the prevalence rate of 46%, 38% and 70% respectively. Further more their distribution as well as density were analysed along with other predator nematodes and free living nematodes.

4.1 Taxonomic treatment of plant parasitic nematodes of grape vine

Taxonomy of plant parasitic nematodes were carried out with morphometric measurement of taxonomically important body parts. The observed data were compared with the published taxonomic key of Smart and Nuygen (1988). The analysis revealed the grape vines of CHC were found infested with *Tylenchorhynchus* sp., *Xiphinema* sp. and *Longidorus* sp.

Table 1: Morphometric Data for species identification.

Linear measurement	*Tylenchorhynchus*		*Xiphinema*		*Longidorus*	
	Mean(N=5)	SD	Mean(N=5)	SD	Mean(N=5)	SD
Body length(mm)	0.58	0.07	2.15	0.55	2.61	1.54
Maximum width(µm)	20.16	1.31	114.28	14.98	79.2	43.56
Oesophageal length(µm)	107.5	1.07	560.34	61.28	471.7	300.87
Oesophageal width(µm)	18.48	1.07	99.36	12.42	71.22	33.74
Distance from head to vulva(µm)	437.4	54.34	1584	509.63	2634	74.79
Body width at vulva(µm)	21.2	0.69	95.52	10.37	103.2	4.8
Length of stylet(µm)	21.36	3.21	14.88	1.81	15.88	1.43
Distance at mouth part(µm)	5.76	1	12.81	1.01	16.32	4.69
Length of tail(µm)	10.8	0.84	78	4.32	46.08	9.95
Diameter at anal part(µm)	8.4	0.84	66.72	4.61	55.2	25.45
a	29.1	3.27	18.62	2.51	32.56	3.45
b	5.45	0.68	3.80	0.59	5.83	0.73
c	54.23	4.31	27.45	5.84	53.6	25
c’	1.28	0.02	1.17	0.02	0.95	0.32
v	68.42	5.39	72.31	6.19	69.84	1.09

a= Body length/ Maximum width b= Body length /Oesophageal length c=Body Length /Tail length c'=Tail length / Body width at anal part v=Distance from head to end of vulva×100/Body length Remaining other parts was measured in 10X×40X i.e (40X objectives)

***Tylenchorhynchus* sp.**

Tylenchorhynchus are economically important plant parasitic nematodes that parasitize a wide variety of plants. In comparison to other two plant parasitic nematodes, *Tylenchorhynchus* sp. are relatively smaller in size. Being a smaller nematode it has comparatively longer tail and stylet. Tail length /Body width at anal part (c'ratio) is also maximum in comparison to other two parasitic nematodes (Table 1)

After fixation these plant nematode turn into 'c'shaped in structure. Beside this, other important diagnostic characters include low lip region, rounded and almost continous to body contour with three to four annules. Clearly visible tail with terminus rounded and clavate in structure as described by Hando et al., (2000).

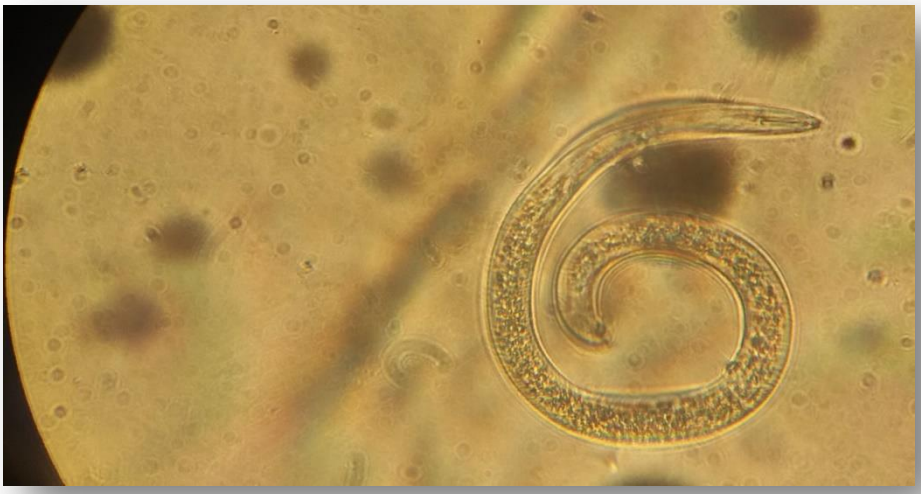

Photo8: *Tylenchorhynchus* sp. (10X×40X)

***Xiphinema* sp.**

Xiphinema sp. are commonly called as dagger nematodes. In comparison to other two plant parasitic nematodes *Xiphinema* sp. has maximum body width and oesophageal width and maximum diameter at anal part. The diameter of anal part is comparable to *Longidorous* but thicker than *Tylenchorhynchus* (Table 1)

These plant parasitic nematodes turns into 'c' shaped in structure (Photo 9) These plant parasitic nematodes posseses longer stylet with flanged shaped at the base (photo 10) oesophagous are bottle shaped in structure (photo 10).Tails are blunt in structure (Photo 11) as shown by Smart and Nuygen (1988).

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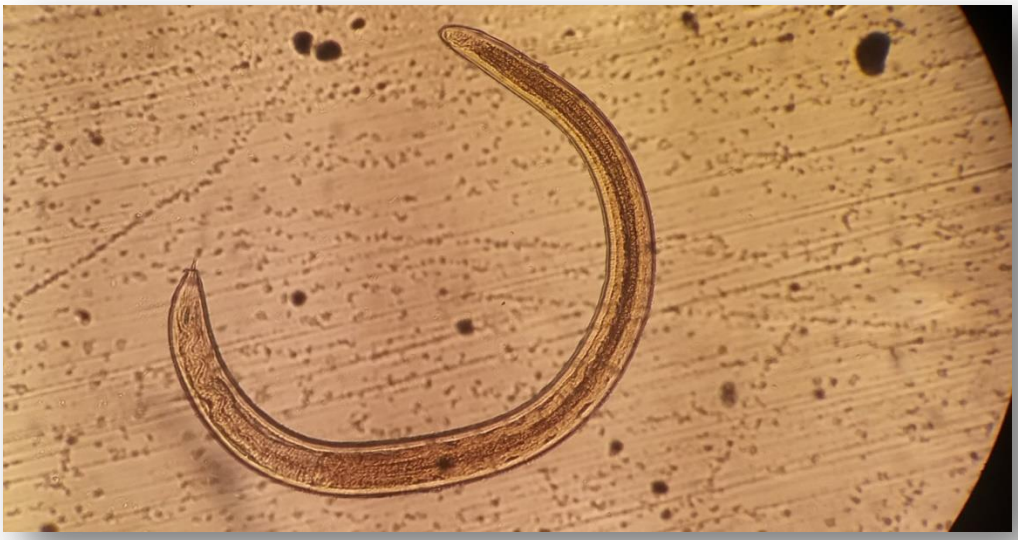

Photo 9: *Xiphinema* sp. (10X×10X)

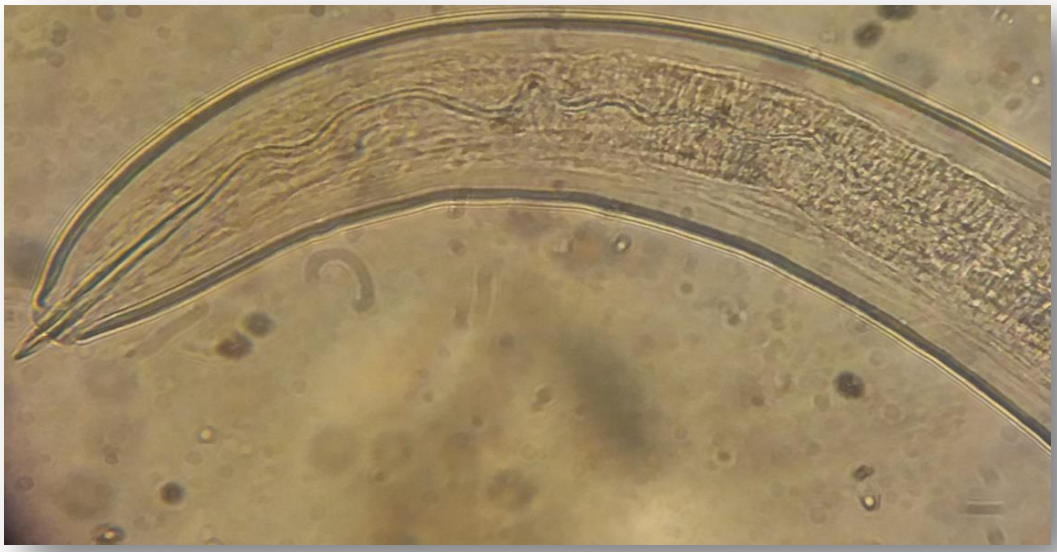

Photo10: Anterior part of *Xiphinema* sp. (10X×40X)

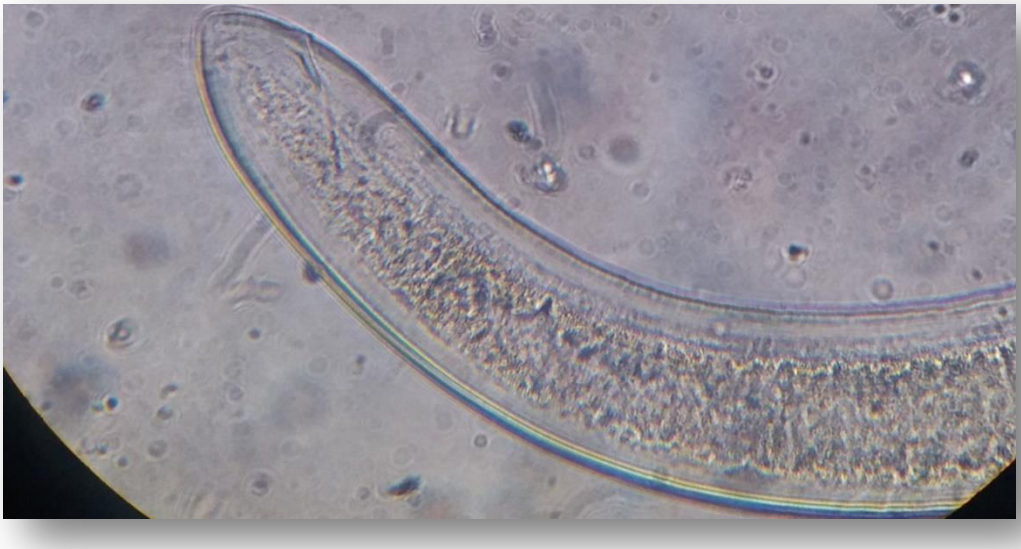

Photo11: posterior part of *Xiphinema* sp. (10X×40X)

***Longidorus* sp.**

Among three nematodes *Longidorus* sp. are the longest nematodes as they are almost straight during fixation (photo12). Oesophageal length is longer than other two parasitic nematodes. Body length /Maximum width (a ratio) is also greater in *Longidorus* sp. It has maximum diameter at mouth part (Table 1). These nematode has elongated and narrow body. An oesophagus is bottle shaped and which has weak musculature (Photo12)

Photo12: Anterior part of *Longidorus* sp.(10X×40X)

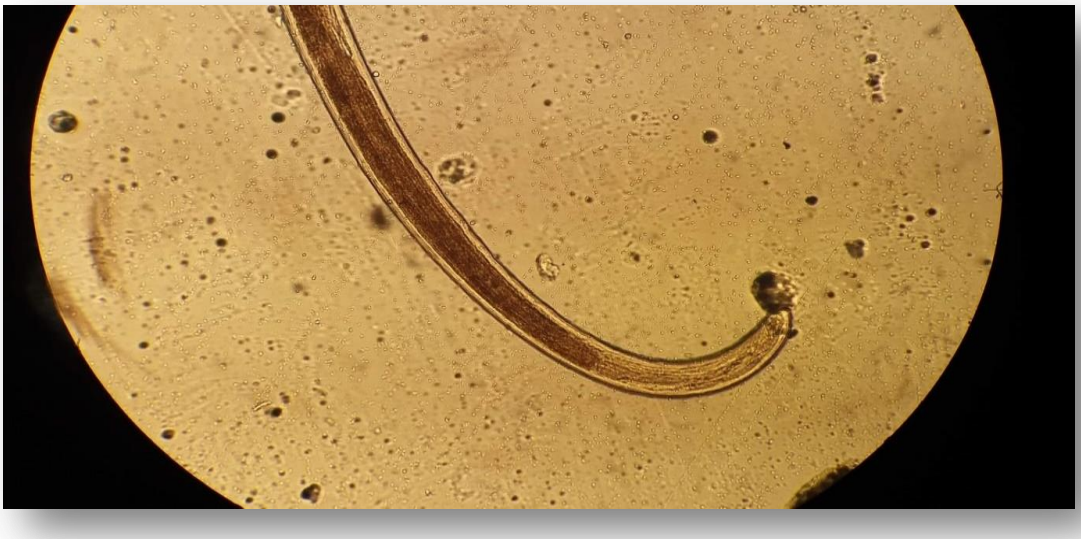

Photo13: Posterior part of *Longidorous* sp. (10X×40X)

4.2 Distribution and density of plant parasitic nematodes.

Season of the year has direct influence on the distribution and density of plant parasitic nematodes that infest the grape vine. Some parasites infest the plant throughout the season while other during specific season.

Table 2:Distribution and density of plant parasitic nematodes

Distribution of plant parasitic nematodes		
Parasitic nematodes	Summer (N=50)	Winter(N=50)
Tylenchorhynchus sp.	0(0%)	23(46%)
Xiphinema sp.	0(0%)	19(38%)
Longidorus sp.	22(44%)	13(26%)
Density of plant parasitic nematodes		
Parasitic nematodes	Summer(N=809)	Winter(844)
Tylenchorhynchus sp.	0(0%)	41(4.8%)
Xiphinema sp.	0(0%)	30(3.5%)
Longidorus sp.	46(5.7%)	16(1.9%)

The result showed that among three plant parasitic nematodes, only *Longidorus* sp. was found to be distributed in both summer and winter season. Both *Xiphinema* sp. and *Tylenchorhynchus* sp showed higher distribution in winter season compared to *Longidorous* during summer season.(Table 2)

Density of the parasite i.e parasitic load around the rhizosphere of grape vine indicated that during summer season only. *Longidorous* species infest the plant with high density while during winter season grape vine are infested with all three nematodes in low density with *Longidorous* sp. compared to rest two species. (Table 2)

4.3Distribution and density of predator and free living nematodes

During the screening of the soil sample of rhizosphere of grape vine, along with parasitic nematodes two species of predatory and other free living nematodes were isolated. Predatory nematodes isolated include *Mononchus* sp. and *Diplogastrine* sp.. While among free living nematodes only rhabditis group was identified.

Table 3: Distribution and density of predator and free living nematodes

Distribution of predator and free living nematodes		
Predator and Free living nematodes	Summer (N=50)	Winter (N=50)
Mononchus sp.	30(60%)	0(0%)
Diplogastrine sp.	29(58%)	36(72%)
Rhabditis sp.	50(100%)	50(100%)
Mesorhaditis sp.	50(100%)	50(100%)
Other free living	0(0%)	15(30%)
Density of predator and free living nematodes		
Predator and free living nematodes	Summer (N=809)	Winter (N=844)
Mononchus sp.	50(6.18%)	0(0%)
Diplogastrine sp.	40(4.94%)	50(5.92%)
Rhabditis sp.	436(53.89%)	442(52.36%)
Mesorhabditis sp.	237(29.29%)	240(2.96%)
Other free living	0(0%)	35(2.96%)

Both predator nematodes were observed in summer season but only *Diplogastrine* sp. were observed in winter season. *Rhabditis* sp. and *Mesorhabditis* sp. were observed in both summer and winter season. Beside rhadbitis group of free living nematodes some other free living nematodes (unidentified) were also isolated only during winter season(Table 3).

Densities of predator nematodes were equal in summer season. While in winter season only *Diplogastrine* sp. nematodes were higher in density. *Rhabditis* sp. and *Mesorhabditis* sp. showed higher density in both summer and winter seasons(Table 3).

Photo Anterior part of *Mononchus* sp. (10X×40X)

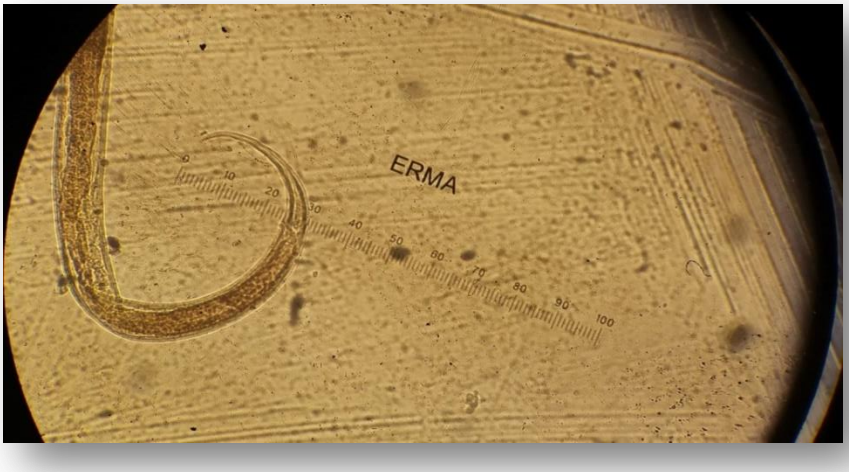

Photo:Posterior part of *Mononchus* sp.(10X×40X)

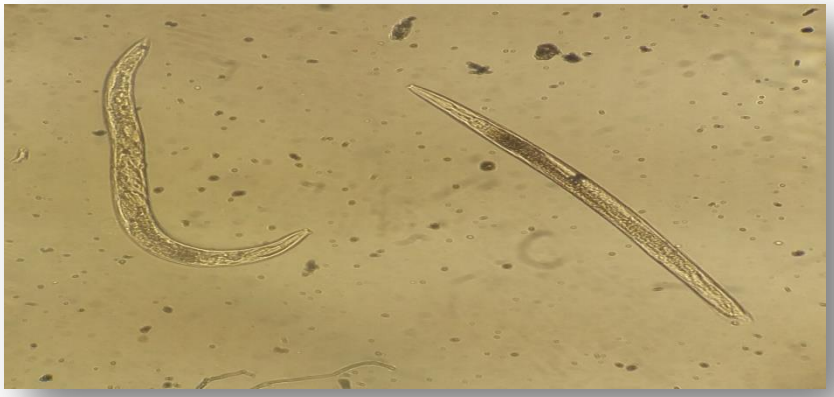

Photo:*Rhabditis* sp.(10X×10X)

5. DISCUSSION

Grape is one of the earliest domesticated horticultural crop in the world which has been widely cultivated for fruit and wine (Mc Governe, 2004). Most grapes are used for wine making (71%) while about (27%) are consumed as fresh fruits and only (2%) are consumed as dried fruits (Cooper *et al.*, 2012). First commercial vineyard was planted in western Oregon in 1962 (Pinkerton *et al.*, 1999). Major grape producing countries are India, Brazil, Venezuela, Peru, Colombia, Thailand, Uruguay, Australia, Mexico, Israel, Egypt (Souza-Leao, 2014). V*itis venifera* had played an important role with respect to economy and culture particulary in Europe and some Asian Countries (Brown et al., 1993).

Grape cultivation was started in Nepal during Rana regime (Dahal *et al*., 2017). Climatic factor, geographical structure, as well as soil structure play important role in cultivation of grape vine. In the context of Nepal mid hilly region are described as suitable for grapevine cultivation (Acharya and Yang, 2015). Commerical grape production started in Nepal at Banke and Bardia in 1987 (Atreya and Manandhar, 2016). *Vitis venifera* were introduced in CHC in 1998/1990 (Thapa, 2012).

Vitis venifera are economically important crop facing many soil borne pathogen and pest that damage or completely destroy new roots of plant (Aballey *et al*., 2009). Grey mould caused by *Boteytis cinerea* is a well known disease which causes heavy loss in a grape production around world (Elad *et al*., 1991). Nematodes are serious threat to vineyards in world wide (Brown, 1993; Anwar and Van Gunday, 1989). Among several parasitic nematode affecting grape vine *Meloidogyne* sp. and *Paratylenchus* sp. as well as *Xiphinema* sp. have been reported from America such as Oregon (Pinkerton *et al*., 1999) as well as Washington (Zasada *et al*., 2012) in states.

Several plant parasitic nematodes was reported i.e *Meloidogyne* sp. (Amatya and Shrestha, 1969) *Pratylenchus* sp., *Tylenchorhynchus* sp., *Helicotylenchus* sp., *Rotylenchus* sp., *Xiphinema* sp., *Longidorus* sp. (Pokharel *et.al*., 1994) *Hemicycliophora* sp., *Heterodera avenae*, *Hirshmanniella* sp. (Upreti, 2002) from different areas of Nepal. This is the first report of plant parasitic nematodes in grape vine of CHC, Kirtipur.

Taxonomically nematode species belonging to three different genus were extracted from grape vine of CHC. Among three nematodes, *Tylenchorhynchus* were smaller in size with 0.58 mm with the longer stylet length which is matched with earlier reports (Hando *et al*., 2000). Body length ranges from 0.42 to 1.60 mm (Handoo, 2000) which are the characteristics features of *Tylenchorhynchus* sp.. *Tylenchorhynchus qasamii* are 'c' shaped after fixation (Ramzan *et al*., 2008). Among three nematodes, *Xiphinema* sp. have maximum width with 114.28 µm, maximum anal part with 66.72 µm. *Xiphinema californis* has a longer odontostylet (Cho and Robbins, 1999). *X. brevicolle* has length of 2.1mm and *X. turcicum* has been recorded with length of 4mm (Lambert *et al*., 1983). Among three nematodes, *Longidorus* were longest nematodes with 2.61mm with longest oseophageal length. *Longidorus* sp. are long ranges from 1.5 mm to 12 mm that infest on plant roots (Hunt, 1993). *Longidorus iranicus* was found in rhizosphere of grape which

has length of 5-7 mm (Sturhan and Barooti, 1983). Lamberti and Agostinelli, (1993) reported *Longidorus raskii* from rhizosphere of apple with the measurement of 7.1 mm.

Stunt nematodes *Tylenchorhynchus* sp. are economically important plant pathogen which contain 111 species that parasitize a wide variety of plants (Handoo *et al*., 2000). About 8% of total known species of *Tylenchorhynchus* sp. may be parasitic to different plant species (Anderson and Potter, 1991). Diffferent species of *Tylenchorhynchus* were found to be parasitic on different types of plant (McSorley, 2013; Zaki and Mantoo, 2003). *Tylenchorhynchus* mainly parasitize graminae family such as rice wheat, maize (Upreti, 2000) which has been reported from both upland and lowlands of Nepal (Pokharel and Regmi, 2000). These nematode parasite not only infest graminae family but also fruit plants such as Cherry, Walnut, Apple, Apricot, Plum and Pear in Kashmir valley (Zaki and Mantoo, 2003). *Tylenchorhynchus claytoni* were present on tobacco root(Mc Intyre and Miller 1976). These nematodes species was reported in vineyard of India (Bhatia and Gupta, 1973) and in Australia (Stirling, 1976).

Tylenchorhynchus mashhoodi has been reported from vegetable crops of Kathmandu valley (Keshari and Gupta, 2016) and also from in Pears (Shrestha, 2015). There is no report of this genera have been found so far in grape vine plants. Thus this may be first report of *Tylenchorhynchus* sp. found infesting in grape vine of CHC, Kirtipur, Kathmandu.

Longidorus nematodes are known to occur at soil depths of atleast a meter (Hunt, 1993). Needle nematode (*Longidorus* sp.) consists of more than 150 valid species (Archidona Yusle *et al*., 2016). *Longidorus* sp. are reported to affect trees which are responsible for severe decline symptoms in trees (Hashmin, 1983). Some species of *Longidorus* sp are economically important pest of agricultural plants and also transmits nepoviruses (Taylor and Brown, 1997). *Longidorus* species are reported to cause stunting and swelling of roots in perennial crop (Guo *et al*., 2011). Various species of *Longidorus* i.e *L. attenuatus, L. elongatus, L. intermedius, L. juvenilis, L. macrosoma* and *L. poesseneckensis* were isolated from rhizosphere of wild growing grapes (*Vitis venifera*) in the riparian woods of river in Austria (Tiefenbrunner and Tiefenbrunner, 2004). Six *Longidorus* sp. are known to transmit nepoviruses associated with grape vine disease (Lamberti and Roca, 1989). The optimum temperature for *Longidorus elongates* range for both survival and development was 20-25°c (Boag, 1985).

In present study, grape vine plant of CHC were found to be infested with 35% of *Longidorus* sp. with high prevalence in summer than winter. Similarly Amatya and Shrestha, (1996) have recorded *Longidorus* sp. from cauliflower, chilly, wheat, paddy, cabbage and tomato. Shrestha, (2015) recorded relatively higher infestation of *Longidorus* sp. in pear tree in summer and autumn sample in Nepal. The population of *Longidorus* sp is generally greater in a soil with greater porosity (Yeates *et al*., 2008).

Xiphinema sp are migratory ectoparasites that move freely in soil and feed from surface of host's root which inhibit root growth and reduce yield (Brown *et al*., 1993). *Xiphinema*

index and *Xiphinema americanum* are pathogenic on grape (Nigh, 1965; Raski and Radewald, 1958) and also vector of nepoviruses that affect grapevine (Hewitt *et al*., 1958). Sasser, (1989) reported the species of *Xiphinema* from different economically important plant such as Banana, Grapes, Sugarcane, *Citrus* from different country of world. Zasada *et al*., (2012) encountered plant parasitic nematode *Xiphinema* sp. from Washington vineyard. Graham *et al*., (1988) reported *X. bricolensis* in a wide range of soil types and associated with grape vine of Okanagan valley in British Columbia. *Xiphinema americanum* were found in 94% of vineyard and 78% of blocks surveyed in Western Oregon (Pinkerton *et al*., 1999). *Xiphinema vuittenezi*, *Xiphinema diversicaudatum*, *Xiphinema pachtaichum* were found to be infested in grape vine of Iraq (Stephan *et al*., 1985). *Xiphinema pachtaicum*, *X. index*, *X. italiae*, *X. inges* were found to be infested in grapevine of Cyprus (Anonjou, 1981). *Xiphinema index* has been reported to increase more rapidly in sandy loam or fine sands under green house condition (Sultan and Ferris, 1991). The period of soil dryness that occur in October may increased *X. index* rate of reproduction which increase population level in winter month (Feil *et al*., 1997). In England population of *X. diversicaudatum* and *X. index* were highest in autumn and lowest in spring (Cotton et al., 1970). Grape vine of CHC were found infested with 19% of *Xiphinema* sp with density of 3.5% *Xiphinema* sp. not only infect grapevine but also infest vegetable (Bhatta, 1967) maize (Sharma –Poudyal, 2004) *Pinus, Alnus, Quercus* trees (Sharma, 2004) apple (Sudershan *et al*., 2002) from Nepal.

Predatory nematodes consume on plant parasitic nematodes which can be used as agent in the biological control of plant parasitic nematode (Khan and Kim, 2007). In CHC, around the grape vine, along with plant parasitic nematodes two species of predatory nematodes, *Mononchus* sp. and *Diplogastrine* sp. were isolated. *Mononchus* sp. nematodes are non parasitic nematodes that feed on protists as well as other small nematodes (Mankau and Mankau, 1963). Predaceous nematode belonging to order Mononchida are predaceous which feed on protozoa, rotifers, algae and nematode too (Bilgrami *et al*., 1986). Predatory nematodes reduce the population of plant parasitic nematodes (Devi and George, 2018). *Mononchus* sp. prefers *Meloidogyne* sp. *Pratylenchus* sp., *Meloidodera* sp. and *Tylenchorhynchus* sp. (Devi and George, 2018). Rama and DasGupta, (1998) reported that *Mononchus* sp. increase when green manure is used in soil.

Zaki and Mantoo (2003) have reported the occurrence of one predaceous species of *Mononchus* in fruit trees in Kashmir valley. Keshari *et al*., (2018) reported *Mononchus aquaticus* from major vegetable crops from Bhaktapur and Kavre districts of Nepal. Chettri and Subedi, (2019) reported *Mononchus* sp. from Kiwi plant, in pear (Shrestha, 2015) but there is no report of genera have been reported earlier from grape vine.

Goodey (1935) have reported two species of *Diplogaster* from bulbs of *Lilium longiflorum* which included *Diplogastrine longicauda* and *Diplogastrine striatum*. They are found to predate on bacteria (Kimpinski and Sturz, 1996) as well as other nematodes such as *Meloidogyne* and *Tylenchus* (Osman, 1988). The optimum temperature for Diplogastrid predator was found to be between 25-30°C, *Diplogastrine stagnalis* preyed maximum at temperatures ranging between 25-30°C (Shafqat *et al*., 1987). *Diplogastrine*

sp. have ability to recognize their own chemical secretion and have ability to attract their own members possibly reduce cannibalism (Devi and George, 2018).

Free living nematodes can be utilized as bio pointers of soil well being (Neher, 2001). Alteration to soil of treated the soil excrement and urea was observed to be helpful in expanding free-living nematodes and predatory nematodes while it reduce parasitic nematodes in soil (Akhatar and Mahmood, 1996). Several free living nematodes were also extracted along with plant parasitic as well as predator nematodes from the soil collected from the rhizosphere of grape vine of CHC. Among them *Rhabditis* and *Mesorhabditis* were only identified. A total of 27 species of free living nematodes were accounted from Vietnam (Gagarin, 2008), 26 species from Himalayas in Nepal (Andrassy, 1978). Nematodes nourishing habbits was given by Neilsen in 1949 (Yeates *et al*., 1993). Dujardin (1845) was first to perceive a relationship of free living nematodes and Plant parasitic nematodes. Free living nematodes are important for plant productivity (Yeates and Coleman, 1982). *Rhabditis* sp and *Mesorhabditis* sp have close association with the parasitic nematode of *Tylenchorhynchus* sp., *Helicotylenchus* sp. and *Meloidogyne* sp. in vegetable farm along with the predator nematodes of *Mononchus* sp. and *Iotonchus* sp. (Keshari *et al*., 2018).

6. CONCLUSION AND RECOMMENDATIONS

Grapevines are the economic plants and have not been commercialized yet in Nepal. Bacterial, viral as well as parasitic diseases can seriously damage the productivity of the plant. The present study was attented to find out the distribution and density of plant parasite as well as other nematodes associated to rhizosphere of grape vine in CHC, Kirtipur, Kathmandu using Seiving and Baermann's funnel. Altogether 7 genus of nematodes were recorded during study period. *Tylenchorhynchus* sp., *Xiphinema* sp. and *Longidorus* sp. were parasitic nematodes. *Mononchus* sp. *Diplogastrine* sp. were Predatory nematodes. While *Rhabditis* sp. and *Mesorhabditis* sp. were free living nematodes. Taxonomical treatments of parasitic nematodes were done. Number of *Tylenchorhynchus* sp. was higher in winter as compared to other nematodes while in summer only *Longidorus* sp. was present. Density of plant parasitic nematodes were found comparatively less than predator and free living nematodes. Presence of three different types of parasitic nematodes infecting grape vine can cause serious damage in future unless they are properly managed timely.

Recommendations

a) An extensive study on the parasitic nematodes in different plant and horticulture production is needed as it may cause a decline in production.

b) Government, semi government, NGOs and INGOs must be involved in setting of well equipped laboratories with necessary chemicals and materials will be cooperative to check farmer's enemy.

c) Farmers are often unaware that nematodes exist. Education must start with fundamentals from basic level to the masters level. Farmer must be educated as to what nematodes are and how to recognize symptoms of nematode damage in their fields.

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