INVESTIGATION FOR POTENTIAL TRAP CROPS TO REDUCE THE OROBANCHE SEED BANK IN INFESTED TOMATO FIELD, LALBANDI, SARLAHI

A THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER'S DEGREE IN BOTANY

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FEBRUARY 2021

DECLARATION

I, *Nabin Kumar Darai*, hereby declare that the work enclosed here is entirely my own, except where states otherwise by reference or acknowledgement, and has not been published or submitted elsewhere, in whole or in part, for the requirement for any other degree or professional qualification. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and listed in the reference section.

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RECOMMENDATION LETTER

This is to recommend that the Master's thesis entitled "**Investigation for Potential Trap Crops to Reduce the *Orobanche* Seed Bank in Infested Tomato Field, Lalbandi, Sarlahi**" is carried out by Mr. Nabin Kumar Darai under my supervision. The entire work is based on original scientific investigations and has not been submitted for any other degree in any institutions. I therefore, recommend this thesis work to be accepted for the partial fulfilment of M.Sc. Degree in Botany.

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LETTER OF APPROVAL

The thesis entitled **"Investigation for Potential Trap Crops to Reduce the *Orobanche* Seed Bank in Infested Tomato Field, Lalbandi, Sarlahi"** submitted to Department of Botany, Amrit Campus, Tribhuvan University by "*Nabin Kumar Darai*", "*5-2-19-438-2010*" has been accepted for the partial fulfilment of the requirement for Master's Degree in Botany.

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ACKNOWLEDGMENTS

First of all, I owe deep gratitude to my supervisor Prof. Dr. Bipana Devi Acharya (pandey), Department of Botany, Amrit Campus, Tribhuvan University for her immense supervision, effortful guidance and valuable suggestions throughout the completion of this research work.

I am grateful to Prof. Dr. Mohan Devkota, Head, Department of Botany and Lecturer Dr. Laxmi Joshi Shrestha Program Coordinator, Amrit Campus, Tribhuvan University, for their valuable suggestions, ever encouraging and motivating guidance.

I am also grateful to. Prof. Dr. Kanta Poudyal, former Head, Department of Botany and Prof. Dr. Mukesh Kumar chettri, former Program Coordinator, Amrit Campus, Tribhuvan University for their valuable suggestions, encouragement and guidance. during field work and data analysis.

My immense thank also goes to University of Grant Commission (UGC) for granting me financial support for this research work.

I am sincerely thankful to my friends Sanju Jyawali for her help and assistances during field visit and laboratory work, Santosh Gautam for data collection and also to other colleagues for their suggestion, help, encouragement and assistances.

I must thank family members of Hem Kumar Shrestha for providing his land for this research work, cooperation and hospitality during field stay. I am also thankful to local people of Newar Tole, Lalbandi-8, Sarlahi for their immense response's during interactions, supports and sharing their information.

Finally, I am greatly indebted to my relatives and family member for their moral support, encouragement and financial support.

Nabin Kumar Darai
February 2021

ACRONYMS AND ABBREVIATION

%	Percentage
°C	Degree Celsius
µ	Micrometer
ANOVA	Analysis of Variance
ATG	Agricultural Technology Center
Avg	Average
$CaCl_2$	Calcium chloride
cm	Centimeter
DAP	Diammonium phosphate
DHM	Department of Hydrology and Meteorology
DMRT	Duncan's Multiple Range Test
E	East
FAO	Food Agricultural Organization
Feb	February
Fig.	Figure
g	Gram
g/cm^3	Gram per cubic centimeter
g/hec	Gram per hectare
g/ml	Gram per milliliter
i.e.	That is
kg	Kilogram
kg/hec	Kilogram per hectare
m	Meter
m^2	Meter square
$MgSo_4$	Magnesium Sulphate
mm	millimeter
N	North
NARC	Nepal Agricultural Research Council

No.	Number
r.p,m	Revolution per minute
Sd	Standard deviation
SE	South East
Sep	September
Spp	Species
SPSS	Statistical Packages for Social Science
Sq.m	Square meter
SW	South West
Tmax	Temperature maximum
Tmin	Temperature minimum
Trt	Treatment
VDC	Village Development Community
w/v	Weight per volume

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ABSTRACT

Broomrapes (*Orobanche*) are holoparasites devoid of chlorophyll and entirely depend upon the host plant for water and mineral nutrients requirements and can only germinate in response to germination stimulants produced from the root exudates of host plants in the warm and dry environment. Broomrape is becoming major and serious threats in the vegetable crops in the warm and dry region. Seed recovery was done from the soil with following method of sieving and differential floatation technique of Ashworth (1976) with some modification (Acharya *et al*., 2003) was used for the *Orobanche*. The standardization of seed was done in four different types of soil namely, sandy, sandy loam, loam and clay soil. The pre-sowing and post-harvest soil sampling were collected by composite sampling method. The recovery percentage of *Orobanche* seeds from different types of soil showed that their recovery varies according to soil types. Highest percentage of *Orobanche* seeds recovery was obtained in sandy soils (69.8±7.26) followed by sandy loam, loam, and clay soil i.e. 64.4±10.74, 64.2±10.89, and 56.8±12.99 respectively. The significant reduction of *Orobanche* seed bank in soil was found in faba bean (*Vicia faba*) and chilli (*Capsicum frutescens*) compared to control field experiment. The highest *Orobanche* seed reduction was found in faba bean (70.29±7.85).

From this experimental study for potential trap crops in polyethylene bag and field clearly showed that crops like faba bean, Chilli ranked highest among the tested crops which reduce *Orobanche* seed bank significantly.

On the basis of results obtained from experimental study in both polyethylene bag and field, the crops investigated could be listed in the following categories: Highly potential trap crops: *Vicia faba* and *Capsicum frutescens*. Moderately potential trap crops: *Trigonella foenum-graecum*, *Foeniculum vulgare*, *Linum usittatisimum*, *Glycine max* and *Pisum sativum*. Non-potential trap crop: *Allium sativum*, *Allium cepa*, *Raphanus sativus*, and *Solanum tuberosum*

***Keywords*:** Broomrape, non-host crop, host crop, quantitative estimation

CHAPTER 1: INTRODUCTION

1.1 Background

Parasitic plants are those plants that depend upon host to survive, and the host plants may or may not survive from their attack. Parasitic plants can be classified into four main groups according to the parts of the host plant they infect i.e. Viscaceae and Loranthaceae (mistletoes) are green hemi parasites that infect aerial parts of shrubs and trees, Cuscutaceae (dodders) are holoparasitic parasites that attack mainly stem of dicot plants, Orobanchaceae (figworts and witchworts) are hemi-parasitic that invade roots of host plants, whereas Orobanchaceae (broomrapes) are holo-parasitic that infect roots of dicot plants (Eizenberg and Goldwasser, 2018). *Orobanche* are economically important species that damages the agricultural fields and forest culture (Sauerborn, 1991a). In sever infestation of *Orobanche* species in cash crops like tomato and brinjal, it may reduce the crop yield up to 100% (Dhanpal *et al.*, 1996; Parker, 2009; Fernandez *et al.*, 2016).

Taxonomically, broomrapes can be classified into two holoparasitic genera i.e. *Orobanche* and *Phelipanche*, based on morphological and karyological criteria and to molecular phylogenetics (Schneeweiss, 2004; Joel, 2009). *Orobanche* species are characterized by unbranched inflorescence and the lack of bracteoles while in *Phelipanche* species, the inflorescence is branched and bear bracteoles (Parker, 2013). Broomrapes are devoid of chlorophyll and entirely depend upon the host plant for water and mineral nutrients requirements (Joel *et al.*, 2013; Westwood, 2013) and can only germinate in response to stimulants produced from the root exudates of host plants in the warm and dry environment (Abbes *et al.*, 2008). Most of the germination stimulants identified so far is plant hormone known as strigolactones which includes orobanchol, didehydroorobanchol and solanacol (Matusova *et al.*, 2005; Yoneyama *et al.*, 2010; Fernández-Apricio *et al.*, 2011; Fernández-Apricio *et al.*, 2016; Screpanti *et al.*, 2016). Some species of Broomrape is becoming major and serious threats in the vegetable crops in the warm and dry region.

1.2 Worldwide distribution

Generally, *Orobanche* and *Phelipanche* species are parasites on a large number of families such as Apiaceae, Asteraceae, Brasicaceae, Fabaceae and Solanaceae which act as host (Goldwasser *et al.*, 1997; Sauerborn, 1991b). There are more than 150 species of *Orobanche* spp.

(Musselman, 1980). But only seven species are regarded as widespread or acute agricultural problems- *O. crenata* Forsk., *O. cernua* Loefl., *Phelipanche ramosa* L. (syn. *O. ramosa* L.), *Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.), *O. minor* Sm., *O. foetida* poir. and *O. cumana* Wallr. (Parker, 2013).

Broomrapes are mostly found in the temperate region of Eastern Europe, Middle East, the Mediterranean region and the Asia (Rubiales *et al.*, 2009). The distribution of broomrapes has been well discussed by Parker (2013). According to him *O. crenata* are distributed around the Mediterranean including North Africa, near East and Western Asia; *O. cumana* is distributed in SE Europe, the Middle East and SW Asia; *O. cernua* is present across Southern Europe, the Middle East, South Asia, Northern and Southern Africa; *O. foetida* is present in Morocco, Tunisia, Algeria, Libya, Spain and Portugal; *O. minor* is present in most parts of Europe, Western Asia, Northern Africa, Ethiopia and Somalia, and also introduced to Japan, New Zealand, Australia and several countries in North and South America. Parker (2013) also explained the distribution of *Phelipanche ramosa* in Europe, Middle East, West Asia, Australia, North Africa, Ethiopia and Somalia; The distribution of *P. aegyptiaca* overlaps with *P. ramosa* in South Europe, the Mediterranean and North Africa but also extends eastwards to South Asia and China.

1.3 Distribution and host range of *Orobanche* in Nepal

There is no accurate data concerning the distribution of *Orobanche* in Nepal. According to Khattri (1997) *Orobanche aegyptiaca* is found in all ecological regions of Nepal from tropical parts of Terai and inner Terai to temperate region up to an elevation of 3800m. The occurrence of *Orobanche* was high in Terai and inner Terai where there is widespread cultivation of mustard and tobacco. It was also common in Kathmandu valley in Kalo Tori (*B. campestris* var. nigra) field near Badikhel area, Godawari. In Nepal, altogether eight species of *Orobanche* are reported. They are *O. aegyptiaca* Pers., *O. alba* Steph. ex Willd., *O. cernua* Loefl., *O. cernua* var cernua Loefl., *O. cernua* var nepalensis Loefl., Steph., *O. ramosa* L. and *O. solmsii* C.B. Clarke ex Hook.f. and *O. coerulescens* Steph (Press *et al.,* 2000).

The *Orobanche* species have their own host specificity but some *Orobanche* spp. parasites more than one species. *Orobanche* attacks twenty-three plants species belonging to seven families of flowering plants in Nepal (Khattri, 1997) (**Table 1.1**).

Table 1.1. The agronomically important *Orobanche* species and their hosts in Nepal

S.N	Hosts	Family	*O.aegyptiaca*	*O.cernua*
1	*Brassica campestries* var.tori L.	Brassicaceae	+++	
2	*B. campestries* var. sarson L.	”	+++	
3	*B.nigra* Koch	”	+++	
4	*B. juncea* Coss. (rai)	”	+	
5	*B. juncea* Coss (rayo)	”	+++	
6	*B. napus* L.	”	+++	
7	*B. oleracea* var. *capitata* L.	”	++	
8	*B. oleracea* var. *botrytis*	”	+	
9	*B.caulorapa* Pasq.	”	++	
10	*B.rapa* L.	”	+++	
11	*B. arvensis*	”	++	
12	*Raphanus sativus* L.	”	+	
13	*Chenopodium album* L.	Chenopodiacea	+	
14	*Vicia faba* L.	Fabaceae	+	
15	*Cicer arietinum* L.	”	+	
16	*Lens esculentum* Medic.	”	+	
17	*Hordeum vulgare* L.	Gramineae	+	
18	*Argemone Mexicana* L.	Papaveraceae		+
19	*Nicotiana tabacum* L.	Solanaceae	+++	+++
20	*Lycopersicon esculentum* Mill	”	++	+++
21	*Solanum melongena* L.	”		++
22	*S.nigrum* L.	”		+
23	*Foeniculum vulgare* Mill	Umbelliferae	+	

(+= rare, ++= common, +++= vary common) (Source: Khattri, 1997)

	
Orobanche emergence on tomato	*Orobanche* emergence on brinjal
	
Orobanche emergence on chilli	*Orobanche* emergence on mustard

Figure 1.1. *Orobanche* emergence on host plant

1.4 Morphology of *Orobanche*

Broomrapes (*Orobanche* spp.) are the angiospermic annual plants which complete lack of chlorophyll, and their size ranges from 10-60 cm in height depending upon the species. The plants are recognized by its yellow to straw-coloured stems, bearing yellow, white or blue, snapdragon beautiful flowers. The leaves are merely triangular scales without chlorophylls. The flowers are developed on the axil of scales (Punia, 2014). The fruit are capsule containing 1200–1500 minute, dark grey seeds which serve as source of infestation in the field. This results in increasing of seed bank making its eradication difficult (Foy *et al.*, 1989 and Acharya, 2012). The seeds of *Orobanche* are very minute in between (0.1-0.35mm) (Joel, 1987; Lopez-Granados and Garciatorres, 1993). It can produce over 100,000 seeds/plant (Gold *et al.*, 1978) and even single *O. crenata* Forks., can produce up to 500,000 seed (Cubero and Moreno, 1979) which can remain viable in the field for up to 20 years in the absence of suitable host plants (Cubero and Moreno, 1979; Puzzilli, 1983).

1.5 Dispersal mechanism

Orobanche seeds are very small. There is various dispersal mechanism by which *Orobanche* seeds can be easily transported from infested field to non-infested field by the help of wind, water, live-stocks, transportation means, global trade, infected agricultural tools, contaminated plant materials (crop seeds and hay), human agricultural practices and contaminated soils and manure (Goldwasser and Rodenburg, 2013; Jacobshon, 1986; Yaacoby *et al.*, 2015). The seeds are not digested and even passing through the alimentary canal so that it can easily infect the field by infected manure. Thus, it is very difficult to control without knowing soil seed bank and its life cycle.

1.6 Life cycle

The life cycle of *Orobanche* includes independent life phase and parasitic life phase (Joel *et al.*, 1995). Independent life phase begins with seed conditioning and germination and lasts until the *Orobanche* attached to the host. Then parasitic life phase starts as soon as a haustorium was developed. At this time, the parasite becomes fully dependent on hosts for water and nutrients. The living tissues of root of the host get penetrated by the haustorium, eventually forming the physiological bridge between the vascular system of the host and that of the parasite (Joel and Portnoy 1998). After few days, parasite shoot gets emerged out of the soil and sets seeds. The metabolic and developmental stage of root parasites is important to develop effective control measures that will specifically prevent the damage to crop field (Joel, 2000).

1.7 Control measures

There are different types of control measures used for the reduction of this problem such as (a) cultural control measures: weeding, sowing date, trap crops and catch crops (b) Chemical control: herbicides, antitranspirants, soil sterilization, allelopathic substances, (c) physical control: soil solarization, (d) host resistance (e) natural enemies of the phytoparasites (Sauerborn,1991a).

1.7.1 Cultural control measures

a. Weeding

Hand weeding is laborious and tedious, and hence it is not encouraging in highly infested regions. It can be done only after their emergence; by that time the host plants are already get affected. In any case, in integrating with different techniques, the seed bank can be reduced effectively with weeding (FAO, 2008).

b. Sowing date

Grenz *et al.* (2005) reported that, deferring the planting date of agricultural crops also influences the germination of *Orobanche* spp. Delaying the ideal date of sowing up to two weeks has reported to decreases the germination of parasitic plant. The sowing date need to be adjusted with local climate and hosts. In *Vicia faba* the planting date moving from October to November, December or January lowers emergence of both *O. crenata* and *O. foetida*, as well as their dry weight.

c. Trap crops and catch crops

The use of trap crop and catch crop is one of the best methods currently available to control agricultural root parasite (Sauerborn 1991a, Acharya *et al.*, 2013). Trap crops are those non host crops which stimulates the seeds of root parasite to germinate but cannot infect host plant and die due to lack of unavailable of host for nutrition (Habimana *et al.*, 2014). Catch crops are usual host crops, which can stimulate parasitic seed germination and infect the host but are harvested before the parasite blooms and develop seeds, thus reduce the seed population in the soil.

d. **Intercropping**

Intercropping is a technique encouraging concurrent crop production and soil fertility building. This method is generally utilized in locales of Africa as a minimal effort innovation of controlling the broomrapes (Oswald *et al.*, 2002). It has been revealed that intercrops with cereals or with fenugreek can decrease *O. crenata* infection on *Vicia faba* and pea due to allelopathic interactions (Fernandez-Aparicio *et al.*, 2007, 2008). This has been confirmed in a subsequent study, in which trigoxazonane was identified in the root exudates of fenugreek which may be responsible for the inhibition of *O. crenata* seed germination (Evidente *et al.*, 2007). Maize and snap bean as potential trap crops on *Orobanche* soil seed bank showed better performance in stimulating germination of *Orobanche* seed bank and raised the germination by 74 and 71 per cent, respectively. Yield of tomato was significantly increased due to the reduction of *Orobanche* seed bank (Abeb *et al.,* 2005).

1.7.2 Chemical control measures

In heavy infested tomato fields, application of sulfosulfuron at 75 g ha^{-1} was effective in preventing the development of broomrape and reducing the seed bank (Dinesha *et al.,* 2012) and was also reported for higher plant height and fruit yield. The herbicides that are currently in use for broomrape control are glyphosate, and herbicides belonging to the imidazolinones (Eizenberg *et al.,* 2006). All of them are systemic herbicides absorbed through foliage and roots of plants with rapid translocation to the attached parasite (Colquhoun *et al.,* 2006).

1.7.3 Physical control measures

Soil solarization method is considered as one of the most effective methods in controlling broomrape in open crops fields (Haidar and Sidahmad, 2000). This technique was used successfully on cropping land in many countries around the world like Middle East with an endemic *Orobanche* problem, as a pre-planting treatment for tomato, carrot, eggplant, *Vicia faba*s and lentils (Mauromicale *et al.,* 2005). The temperatures of 48-57ºC kill *Orobanche* seeds that are in the imbibed state, accordingly, soil should be wet at the time of treatment. Seeds of *O. ramosa* can survive 35 days at 50ºC in dry air, but are quickly killed by temperatures of 40ºC when wet. Therefore, soil solarisation technique is suite for organic farming or other low-input agricultural systems (Ashrafi *et al.,* 2009).

1.7.4 Host resistance

With tobacco cultivars or *Nicotiana* species, little success has been made in recognizing the source of resistance to *Orobanche* based on low induction of germination. In legumes, resistance to *O. crenata* associated with low induction of parasitic seed germination has been reported in several species (Pérez-de-Luque *et al*. 2005), although it is not known whether this is based on production of germination inhibitors or on reduced production of stimulants. Resistance to broomrapes is a multi-faceted response in faba bean and legumes. Several defense mechanisms have been detected in plants resistant to broomrape attack, mainly involving cell wall reinforcement (Perez-de-Luque *et al*., 2005, 2007).

A novel chemical control strategy has been developed during the past few years: systemic acquired resistance (SAR). SAR can be induced in plants by the application of chemical agents. Recently it has been shown that SAR of host plants can be used for the control of important broomrape species (Sillero *et al.,* 2012).

1.7.5 Natural enemies of the phytoparasites

Like any remaining plants, broomrapes have common foes, which can influence their development and can be conceivably utilized as specialists for their control. This strategy uses living beings (insects, microbes and so on) to restrict or minimize broomrapes infestation (Habimana *et al.,* 2014). The 'bioherbicide approach' utilizes harmful strains of microorganisms (infections, microbes or parasites) which normally happen on the weed and improves their dangerous action. The contamination develop of the microbe is controlled to the degree of making huge harm the parasitic weed. Microbes can be utilized as sole specialists or as a component of a complex incorporated control technique (Sauerborn *et al.,* 2007).

1.8 Study of seed bank and its importance

There are several methods of seed bank study. In the method according to Ashworth, (1976), the seed bank can be studied by using sieving and differential floatation techniques using 40% (w/v) calcium chloride of specific gravity 1.3957. Another Acharya *et al.,* (2003); Khattri, (1997), modified this method by avoiding the use of instruments likes centrifuge, millipore filter etc. to isolate *Orobanche* seeds from heavier organic particles using of the equipment were compensated with floating the mixture containing *Orobanche* seeds and organic particles held on 100µ sieve two times in $CaCl_2$ solutions. Another method according to the Visser and Wentzel, (1980); Kachelriess, (1987), was followed by mixing 4 g of soil with 20 ml of $MgSO_4$ solution with a specific gravity of 1.16 g/ml. This mixture was shaken for 90 second and then centrifuged at 2500 r.p.m. for 5 minute. This allowed the soil particles to deposit while the seeds remained floating. The supernatant was retained in meshed filter paper and the seeds were then counted under the binocular microscope. The seeds not recovered were probably lost in the soil aggregate during centrifugation (Ashworth, 1976). The total number of the seeds in the soil samples was calculated by a correction factor based on the percentage of seeds recovered (Lopez Granados and Garcia Torres, 1993).

So for the control of its effects on different crops, soil seed bank study is necessary and feasible method as it can be conducted in any season. Study of seed bank in the infested field is found to be more reliable index of infestation than visual field surveys only (Sauerborn *et al.,* 1991).

Demographic studies may also be used to estimate the economic thresholds (Mortimer, 1983). The demographic phases and parameters used to define the populations depend on the weed species.

1.9 Justification

Orobanche is a serious problem over the world and also a major problem in Nepal especially in Terai region. The region is highly susceptible to *Orobanche* spp. because the area is rainfed with warm and dry climate. These types of environmental condition are favorable for the germination of the parasite. The parasites only germinate when there is availability of chemical compounds like strigolactole or *Orobancheol*, which is found in host and non-host plants (Fernández-Apricio *et al.,* 2016; Screpanti *et al.* 2016, Yoneyama *et al.,* 2013). At Lalbandi, it is a major problem of the farmers and found that 120-130 *Orobanche* shoot were recorded in 1 sq. meter in tomato field (personal communication of NARC people and farmers). They are economically dependent on tomato, brinjal cultivation for livelihood. Lalbandi of Sarlahi district is heavily infested by *Orobanche* species in cash crops like tomato and brinjal, which may reduce the crop yield upto 95% in severe cases (Rubiales *et al.,* 2009). Due to this they are facing high economic loss, thus by finding potential trap crop, can help farmers to reduce the problems of *Orobanche* infestation by reducing the seed bank in the field. The present study has been proposed to identify potential trap crops to reduce *Orobanche* seed bank through cultural method. In Nepal such research work had been carried out in Brassicaceae family but not in tomato. Therefore, this research work has been conducted to evaluate potential trap crops that can reduce the *Orobanche* seed bank in infested tomato fields in Lalbandi. The outcome of this research is helpful for tomato-growing farmers of the study area for the reduction of *Orobanche* problems in a growing season.

1.10 Research question

- Which non-host crops produce root exudates effective to stimulate suicidal germination of *Orobanche* seeds and helps in reduction of seed bank in the infested tomato field?

1.11 Objectives

1.11.1 General objective

- To evaluate the potential trap crops that can reduce the soil seed bank of *Orobanche* infested tomato field.

1.11.2 Specific objectives

- To identify suitable non-host crops as potential trap crop.
- To estimate pre sowing and post-harvest soil seed bank in infested field.
- To study the relationship between cropping pattern and emergence of *Orobanche* in the infested fields.

CHAPTER 2: LITERATURE REVIEW

Orobanche and *Phelianche* species are root holoparasitic plants, depending entirely on their hosts for all of their nutritional requirements and causing severe damage to economically important dicotyledonous plants (Hershenhorn *et al.*, 2009). This parasitic weed requires host plant to complete its life cycle and produce large number of seeds per plant and remains viable for 20 years in soil in absence of host plant (Musselman, 1980; Parker and Riches 1993).

According to Sauerborn (1991a), some particularly damaging *Orobanche* species occur in mediterranean region and in western Asia and parasitize important cash and food crops. *O. aegyptiaca* and *O. ramosa* attack mainly legumes and solanaceae, such as eggplant, potato tobacco, tomato etc. *O. crenata* and *O. minor* parasitize preferentially grain and fodder legumes while *O. cumana* causes great damage in sunflower. Lentil can be parasitized mainly by two different species of broomrapes, namely Crenate broomrape (*Orobanche crenata* Forsk.) and Egyptian broomrape (*Phelianche aegyptiaca* (Pers.) Pomel). *O. cernua* typically attacks Solanaceous crops, especially tomato, eggplant and tobacco and, less commonly, potato.

According to Parker (2013), seven broomrape species belonging to the *Orobanche* and *Phelianche* are considered to be of agricultural importance as crenate broomrape (*O. crenata*) is a major plant parasite that infest a wide range of crops, mainly species belonging to the Fabaceae and Apiaceae and some hosts belonging to the Curcurbitaceae, Solanaceae, Lamiaceae, Ranunculaceae and Asteraceae. Small broomrape (*s. minor*) infest a very wide host range, including species of the Fabaceae, Solanaceae, Ateraceae and Apiaceae. *O. minor* is an important pest in clover and lucerne seed production but has a lesser impact on other susceptible crops. Foetid broomrape (*O. foetida*) attacks a wide range of species restricted to the Fabaceae. It has been reported to be a problem in *Vicia faba* cultivation and also, but to a lesser extent, in chickpea and vetch crops in Tunisia. Sunflower broomrape (*O. cumana*) is specific to sunflower alone affecting the production of oil and seeds. Nodding broomrape (*O. cernua*) attacks mainly Solanaceae crops; it constitutes a severe problem in tomato. Branched broomrape (*P. ramosa*) affects an extremely wide range of host crops belonging to the Solanaceae, Brassicaceae, Cannabaceae, Fabaceae, Apiaceae and Asteraceae. High yield losses have been reported mainly in tomato, tobacco and rapeseed. Egyptian broomrape (*P. aegyptiaca*) has a similar host range to branched broomrape but infests a wider range of Brassicaceae and Curcurbitaceae species. Severe damage has been reported to tomato, potato, lentil, and carrot crops.

Press *et al.*, (2000), reported eight species of *Orobanche* in Nepal namely *O. aegyptiaca* Pers., *O. ramosa* L., *O. cernua* Loefl., *O. cernua* var. Nepalensis Loefl., *O. cernua* var. cernua loefl., *O. solmsii* C.B. Clarke ex Hook.f., *O. alba* Steph.ex Wild. and *O. coerulescens* Steph. *Orobanche* have their host specificity. It attacks twenty-three plant species belonging to seven families (Brassicaceae, Solanaceae, Fabaceae, Umbelliferae, Chenopodiaceae, Papaveraceae and Poaceae) of flowering plant in Nepal (Khattri, 1997). Out of eight, two species *Orobanche aegyptiaca* Pers. and *O. cernua* Loefl., (*O. solmsii*) were causing severe threat to wide range of important vegetables and other field crops, particularly mustard (Brassica campestris), rayo (B. juncea), tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana tobaccum*) grown in Terai and dun valleys (Khattri *et al.,* 1991). *O. aegyptiaca* attacks all above mentioned crops while *O. cernua* are more susceptible to solanaceous crops (Khattri *et al.,* 1991).

AI-Menoufy (1989) and Kleifeld *et al.* (1994), studied trap crops for various *Orobanche* and *Phelianche* spp. Trap crops for *O. crenata* were sorghum (Sorghum vulgare), barley (*Hordeum vulgare*), vetch (*Vicia vilosa* var. dasycarpa) and purple vetch. (*V. atropurpurea*), clover (*Trifolium alexandrinum*), flax (*Linum usitatissimum*), and coriander (*Coriandrum sativum*). Trap crops for *O. cernua*, *O. aegyptiaca* and *O. ramosa* were pepper (*Capsicum annuum*), sorghum (*Sorghum bicolor*), cowpea (*Vigna unguiculata*), hemp (*Hibiscus subdariffa*), mungbeans, (*Phaseolus aureus*), flax, alfalfa (*Medicago sativa*), soybean (*Glycine max*), vetches (*Vicia* spp.) and chickpea (*Cicer arietinum*).

According to the Babaei *et al.*, (2010), sesame, brown Indian hemp, common flax and black-eyed pea decreased broomrape biomass by 86, 85.3, 75.2 and 74.4% respectively. Reducing broomrape biomass caused increases in the tomato yield. Kleifeld *et al.*, (1994), observed that growing common flax (*Linum usitatissimum*) in pot in two successive winter seasons or one summer cropping with mungbean (*Phaseolus aureus* Roxbg.) reduced the early infestation of *Orobanche aegyptiaca* and significantly increased tomato vigor and production. But the *O. aegyptiaca* attacked clover (*Trifolium alexandrinum*), flax and mungbean and developed inflorescences. Various sources of the parasite showed different degrees of virulence to flax, from heavy attack and severe damaged to sparse attachment with no production of flowering shoot. The virulent species attacked flax all year round, causing severe damages. Similar result was reported by Goldwasser *et al.,* (1991), that the flax was heavily parasitized by *O. aegyptiaca* and therefore it was considered as catch crops in those studies. But flax has been cited as an efficient trap crop for *O. crenata* Forks. (Ibrahim *et al.,* 1973), *O. cernua* Loefl. (Krishnamurty *et al.,* 1977) and *O. ramosa* (Hameed *et al.,* 1973

Aksoy *et al.,* (2016), has reported the broccoli of Brassicaceae family, can reduce by 48% and 39%, flax by 52% and 72% in shoot number and 55% and 26% in dry weight of *Orobanche crenata* in the first and second year, respectively. Similarly, study conducted by Arslan and Uygur, (2013), under laboratory conditions in Turkey regarding the effect of the root exudates of flax and lentil on the germination of broomrape seeds, flax as a trap plant was found to highly improve the germination of broomrape. Flax as a trap plant was found to be effective tin capturing *O. crenata* for the first time with this study conducted a lentil field in Adana province. It concluded that growing flax as a trap crop or lentil as a catch crop two months before the sowing of lentil as a crop can be a main element of integrated broomrape management which reduce the soil seed bank of crenate broomrape.

According to Bista (2015), the germination percentage was found to be highest (48.31%) in normal soil moisture condition and lowest (4.6%) in flooded soil moisture condition. This indicates that *Orobanche* seeds are unable to survive for a long period in water logged conditions. According to Parker and Riches, (1993) flooding of rice cropping throughout the growing season destroys *Orobanche* seed. In response to stimulant pH, *Orobanche solmsii* seeds showed significantly a high germination percentage (65.27%) at pH value 6.5 and it declined progressively with the increase of acidic and alkaline conditions. However, seeds appeared to be more sensitive to alkaline rather than acidic condition. The suitable temperature for conditioning of *Orobanche* seeds are between 15-20 ºC for at least 18 days for maximum germination. However, prolonged storage in these conditioning causes the seeds to enter secondary dormancy. Elevating the storage temperature increase the percentage of seeds going dormant, but there is also some decrease in viability at higher temperature, with viability is zero reaching at 80 ºC. (Mauromicale *et al.,* 2000)

Bista (2014), has been reported the diminish seed germination of *Orobanche solmsii* by 43% and 41%, separately with application of ammonium nitrate and ammonium chloride and were more than some other ammonium salts. The level of broomrape seed germination was diminished with increment of nitrogenous manure. Habimana et al. (2014) also reported that elevated level of nitrogen manure suppresses *Orobanche* development and expands crop yield.

Kleifeld *et al.* (1994) justified the importance of using 'trap crops yielding suicidal germination' as a management option for reducing *Orobanche* seed bank in the infested fields. The seed bank of this harmful weed in soil can be decreased by using trap crop. But all the seed bank reduction is not due to trap crop only; some reduction is also contributed by edaphic and pathogenic factors. There are some reports that the crops rotation with certain non- host crop can also reduce *Orobanche* seed bank in the infested field (Sauerborn 1991; Acharya 2013).

Acharya, (2012), has been studied in pot and field conditions to evaluate effects of non-host crops on *Orobanche* seed bank. Pot and field experiments were conducted in the soil naturally infested with *Orobanche* seeds in Nawalparasi district of Central Nepal. Altogether, 21 different non-host crop species were tested in the study. *Orobanche* seed density in soil samples collected from pot/plot before sowing and after harvest of each crop species was recorded. Data of pre-sowing and postharvest were compared in order to assess the effects of the test crops on *Orobanche* seed density. On the basis of degree of effects on the *Orobanche* seed bank, the investigated crop species could be classified in to three categories: (a) non-potential trap crop: garlic, chilli, coriander, carrot, buckwheat, sunflower, french bean, pea, eggplant, potato, fenugreek, wheat and faba bean; (b) moderately potential trap crop: barley, onion, chickpea and maize; and (c) highly potential trap crops: radish, lentil, linseed, fennel and cumin.

The research work was carried out in the Central Rift Valley in 2002 to 2003 in field experiment on naturally *Orobanche* infested soil to test potential of trap crops in *Orobanche* seed bank exhaustion preceeding tomato field. Maize and snap bean showed better performance in stimulating germination of *Orobanche* seed bank and raised the germination by 74% and 71% respectively. Maize and snap bean were also complementing each other under inter-cropping and soil seed bank of *O. ramosa* and *O. cernua* was depleted by 72.5% per season. Yield of tomato was significantly raised due to the reduction of *Orobanche* seed bank in the 3rd season (2004) (Abebe *et al.*, 2005).

Schnell *et al.,* (1994) demonstrated that, the *Vicia faba* was most effective for the *Orobanche crenata*, which reduced the seed bank by (47.3%) and fallow (10.6%) the least effective. Lopez-Granados and Garcia –Torres (1991) also found decrease in number of *Orobanche crenata* shoots on *Vicia faba* with strong increase in the seed content in the soil and Aalders and Pieters (1987) also observed a positive correlation between the biomass of *Orobanche crenata* and the biomass of *Vicia faba*.

Hershenhorn *et al.,* (1996) observed that more than 50% of Egyptian broomrape seeds germinated in the presence of all pepper variety roots except the sweet pepper Maor. However only a few attached and developed. The two paprika varieties Lehava and Shani were hosts to more parasites than the sweet pepper varieties, and showed as many as 15 parasites per host plants. In contrast, tomato roots induced less than 10% seed germination but were highly susceptible to the parasite (30 parasites per host plant). Damage caused to pepper foliage and fruit was not significant, compared to the damage caused to tomato. Pepper roots stimulated germination of 22-26% of nodding broomrape (*Orobanche cernua*) seeds without any parasitic attachments whereas tomato roots stimulated germination of less than 10% of seed but the plants were heavily damaged by parasites. Jaconbshon *et al.*, (1991) had also mentioned that root exudates of pepper stimulate the seed germination of broomrapes without any infection. Sweet pepper has been reported as trap crop for Egyptian broomrape (Bischof and Koch, 1974). Beneficial effect was observed in pot experiments but not observed in field experiment.

According to Zhang *et al.*, (2013), the allelopathic effects of soybean on sunflower broomrape. Fourteen common soybean cultivars were grown in pots. Samples were collected from soybean plants and rhizosphere soil at five growth stages (V1, V3, V5, R2, and R4). The allelopathic effects of soybean reached highest at the V3 stage. Methanolic extracts of soybean roots induced higher broomrape germination than methanolic extracts of stems or leaves. The germination rates induced by root extracts (10-fold dilution) were positively correlated with germination rates induced by stem (10-fold dilution) and leaf extracts (10-fold dilution). The broomrape germination rates induced by root extracts were also positively correlated with soybean nodule diameter and dry weight. The results indicated that soybeans could induce sunflower broomrape germination and can be used as potential trap crop. Intercropping soybean with maize (*Zea mays* L.) can reduce the parasitism rate of *Striga hermonthica* (Del.) Benth, resulting in increased maize yield (Odhiambo, *et al.*, 2011).

Abbes *et al.* (2008) recognized flax, bread wheat, sunflower, oats, durum wheat and fenugreek as trap crops after observing *O. crenata* seed germination on their roots in vitro, but no tubercle formation was observed on roots of any plant after 67 days from inoculation. Al-Menoufi (1989); Bakheit *et al.*, (2002), reported a reduction in *O. crenata* infestation when *Vicia faba* was intercropped with fenugreek. Also, Al-Menoufi *et al.* (1996) indicated the possibility of intercropping lupin, coriander and *Brassica rapa* with *Vicia faba* or tomato to reduce *Orobanche* infestation. Among the cereal crops, oats appeared to reduce *O. crenata* infestation in *Vicia faba* according to Fernández-Aparicio *et al.,* (2007). Fenugreek was further investigated by Fernández-Aparicio, (2008), who observed 30-41% reduction in emerged number of spikes when fenugreek was intercropped with Giza blanka in Egypt.

FernandezAparicio, *et al*., (2007) showed that *O. crenata* attacks on legumes plants and infection on *Vicia faba* and pea reduced when these host crops are intercropped with oat. The number of *O. crenata* plants per host plant decreased as the proportion of oats increased in the intercropping. Pot and field experiments confirmed the reduction of infection in *Vicia faba* intercropped with cereals. It is suggested that inhibition of *O. crenata* seed germination by allelochemicals released by cereal roots is the mechanism for reduction of *O. crenata* infection.

Yield loss due to *Orobanche* is estimated up to 49% in mustard (Khattri, 1997), about 30-40% in the tomato fields of Lalbandhi, Sarlahi district (personal communication with NARC officials and Farmers). The yield loss in solanaceous plants like potato, brinjal, tomato and tobacco by *O. ramosa* L. is reported to more than 75% in Algeria (Kamel, 2005). Lentil can be parasitized mainly by two different species of broomrapes, namely Crenate broomrape (*Orobanche crenata* Forsk.) and Egyptian broomrape (*Phelianche aegyptiaca* (Pers.) Pomel) and yield losses up to 95% have been reported depending on the severity of the infestation and the planting date (Sauerborn, 1991b; Rubiales *et al.,* 2009). Economic loss of about 60 million Euro is estimated annually (Uludag and Demirci, 2005). In the Middle East, the annual yield losses due to these parasitic weeds are estimated to be worth 1.3-2.6 billion dollars (Aly, 2007).

Current methods for controlling parasitic weeds focus on reducing the soil seed bank, preventing seed set and inhibiting seed movement from infested to non-infested areas (FernandezAparicio *et al.,* 2008; Rubiales *et al.,* 2009). The use of catch crops (Acharya *et al.,* 2002) and trap crops (Sauerborn, 1991b) has been suggested as tools to reduce broomrape in infested soil. Currently, there is no consistent and sustainable method for the control of *Orobanche* elsewhere in the World (Parker and Riches, 1993). Use of trap crop is one of the best methods currently available to control agricultural root parasites. Trap crops also called false host which stimulate the germination of the parasite seed but cannot be infected and thus reduce the seed population in the soil. Some authors suggested trap crops as sustainable and useful method for the control of *Orobanche* species (Puzzili, 1983; Labrada and Perez, 1988). The effect of trap crops on parasite plays great role in stimulating seed germination but do not attack themselves by parasites. The use of trap crop in tomato fields in Nepal have not been investigated so far.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study area was Lalbandi municipality, ward no.8 which is situated in central development of Nepal. It is located at 27° 3′ 30″ N to 85° 38′ 0″ E. The district lies about 170 km South-East of Kathmandu. Average elevation of this area is 154 meter above sea level. Now it comes under the province no.2. This area is famous for tomato cultivation and supply it to whole country.

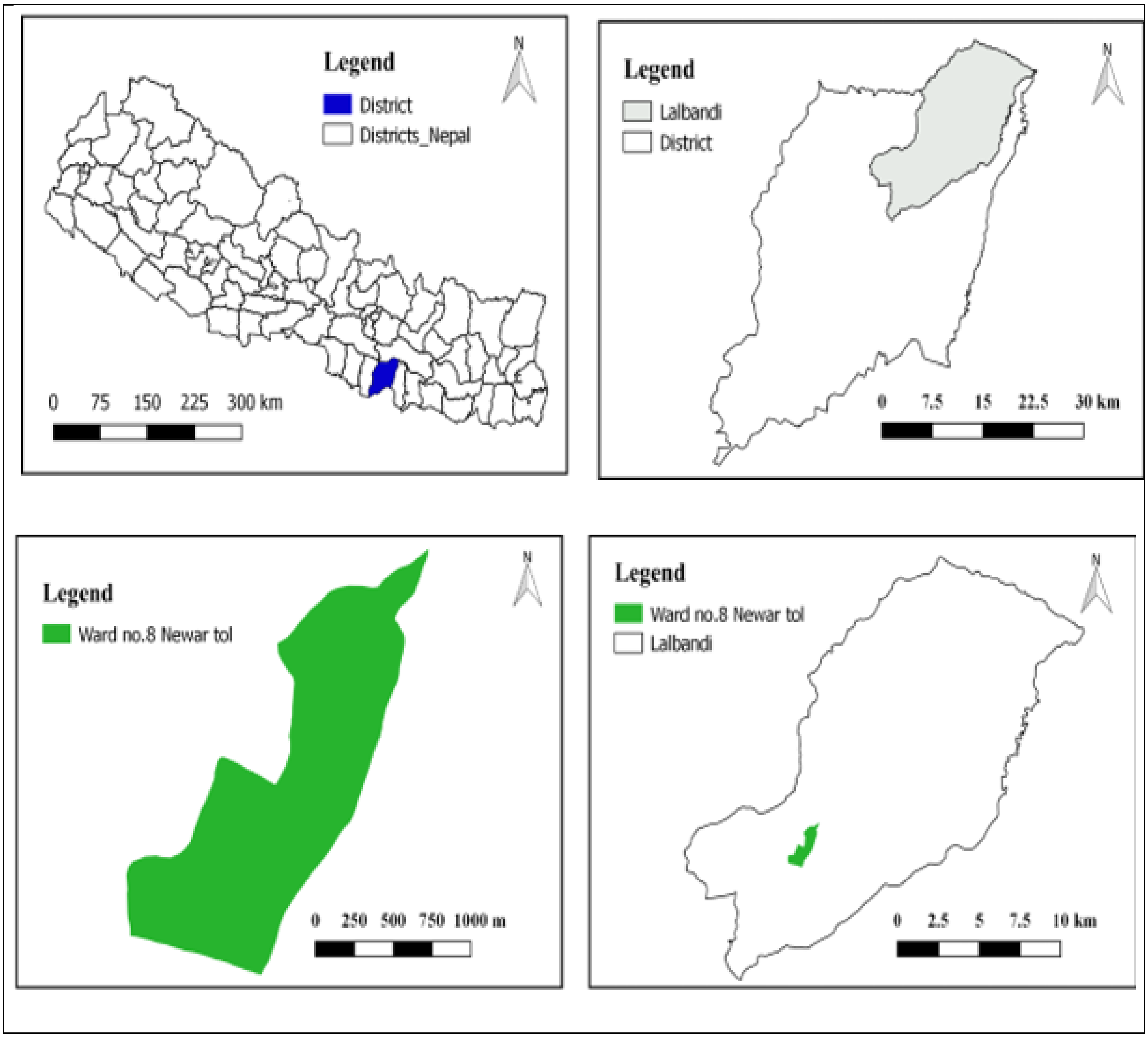

Figure 3.1. Map of study site showing Lalbandhi, Sarlahi, Province 2

3.2 Climate

The climatic data of Karmaiya station was considered for the present study, as this station lies near the study area. Average maximum and minimum temperature recorded in Karmaiya station were 35.4 °C and 8.17 °C respectively. Annual precipitation of Karmaiya station was 1330.9 mm. Maximum precipitations in the month of August 355.8 mm and No precipitation in the month of November and December of 2018 and January of 2019.

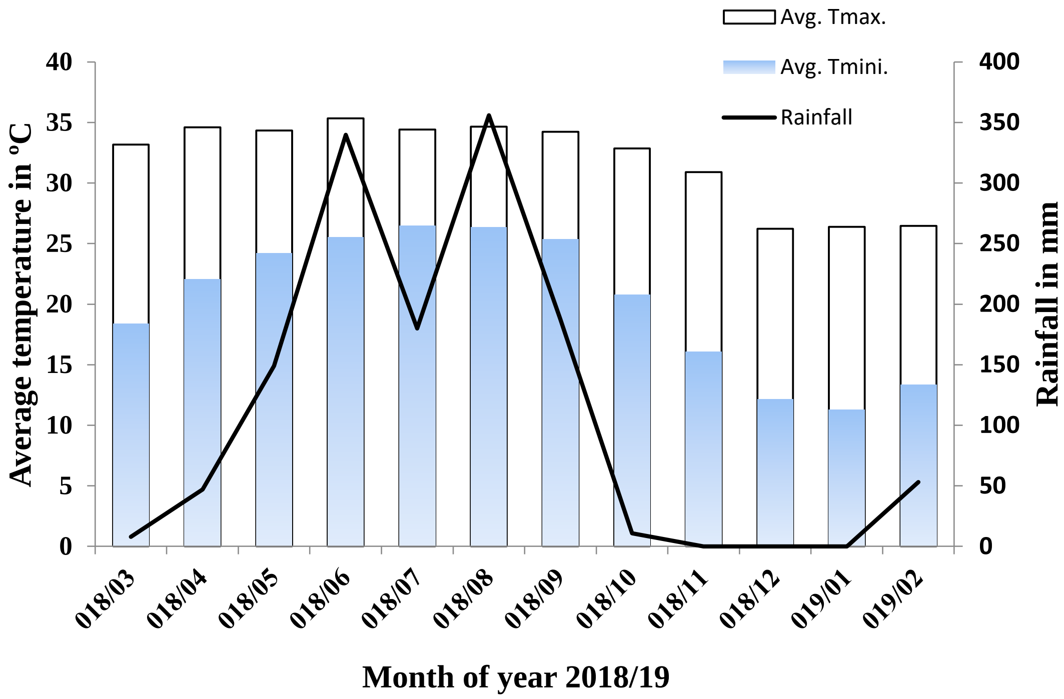

Figure 3.2. Climatic data of study area (Source: Department of Hydrology and Meteorology (DHM))

3.3 Site selection and Field design

Orobanche infested tomato field at Lalbandhi, Sarlahi has been selected for the study. Altogether 11 non host crops, which were grown locally, have been selected for trap crops experiment and fallow as control treatments. Trap crops experiment was conducted in polythene bags and fields plots. The total plot area was 12 x 8.5 m^2 and each plot is 2m X 1m (**Figure. 3.3**)

1 1	2 2	3 3	4 4	← **Plot number** ← **Treatment number**
8 7	7 4	6 6	5 5	
9 F	10 10	11 9	12 8	
13 11	14 F	15 10	16 5	
20 6	19 9	18 7	17 11	
21 2	22 3	23 1	24 8	
25 5	26 3	27 7	28 6	
32 11	31 2	30 9	29 1	
33 F	34 4	35 10	36 8	

Trt. No.	Scientific Name
1	*Linum usitatissimum* L.
2	*Foeniculum vulgare* Mill.
3	*Solanum tuberosum* L.
4	*Trigonella foenum-graecum* L.
5	*Glycine max* (L.) Merr
6	*Allium cepa* L.
7	*Capsicum frutescens* L.
8	*Vicia faba* L.
9	*Allium sativum* L.
10	*Raphanus sativus* L.
11	*Pisum sativum* L.
F	Fallow

Figure 3.3. Outline of experimental field sample (Randomized Complete Block design) and list of test crops

3.3.1 Field history

The study site was rain fed area with irrigation facility by electric motors and the field was planted by maize in preceding season. The selected field was cultivated with tomato (*Solanum lycopersicum*) and brinjal (*Solanum melongena*) in winter and maize (*Zea mays*) in summer for last ten years. The soil Nitrogen (N), Phosphorus (P) and Potassium (K) were as follows 0.09%, 35.02kg/ha and 455.6 kg/ha respectively and Organic matter is also low (1.02%). This indicates that study site was very low nitrogen contents, whereas phosphorous and potash is in medium and high condition. The soil texture of study site was loamy with 47.8% sand, silt 43.4%, and clay 8.8%. The pH of soil 7.5 which indicates slightly alkaline (Soil analysis was done in Agriculture Technology Center, Jwagal, Lalitpur). Manuring was done with animal dung along with mustard oil cakes (at the rate of 2kg per150 sq. m) and DAP (at the rate of 1kg per 150 sq. m).

3.3.2 Field experiment and Plantation of test crops

The field was ploughed twice with tractor with disc harrow two weeks before final preparation i.e. on the 1st week of October. The plots were laid with randomized complete block design with 12 treatments and three replications including fallow as control. The size of each plot was of 2m x 1m and Crops seed were sown 3-4cm deep in the soil. Bulbs of onion (*Allium cepa*), bulb-let of garlic (*Allium sativum*), seedling of chilli (*Capsicum frutescense*) were planted in the field plots. Number of seeds sown and plant maintained in each field plots were given in (**Table 3.1)**

Table 3.1. Name of crops planted, distance maintained between the rows and between the plants during field experiments.

Test crops	**Common**	**Distance between**	
Botanical name	**Name**	**rows(cm)**	**Plants (cm)**
*Linum usitatissimum**	Linseed	25	5
*Foeniculum vulgare**	Fennel	30	5
*Solanum tuberosum******	Potato	30	20
*Trigonella foenum-graecum**	Fenugreek	25	5
*Glycine max**	Soya bean	30	10
*Allium cepa*****	Onion	20	10
*Capsicum frutescens***	Chilli	30	10
*Vici faba**	*Vicia faba*	30	10
*Allium sativum****	Garlic	20	10
*Raphanus sativa**	Radish	25	5
*Pisum sativum**	Pea	25	5

*Seed, **Seedling, ***Bulb-let, ****Bulb, *****Tuber

3.3.3 Polyethylene bag Experiment and Plantation of test crops

Polythene bag experiment was conducted on study site during October 2018 to February 2019 to study the effects of trap crops on seed bank of *Orobanche* spp. For this experiment 11 crops and fallow (as control) were tested. These experiments were performed in black polyethylene bags of 10×12 inch. The polyethylene bags were filled with mixture of soil and manure from the same *Orobanche* infested tomato fields. Each bag was perforated for the aeration and drainage. Three fourth of bags were buried with soil to avoid rapid fluctuation of temperature and moisture.

Seeds or seedlings of test crops were collected from the Kathmandu and Lalbandi local market. Crops seed were sown 3-4cm deep in the soil. Bulbs of onion (*Allium cepa*), bulb-let of garlic (*Allium sativum*), seedling of chilli (*Capsicum frutescense*) were planted in the bag. Number of seeds sown and plant maintained in each bag were given in **Table 3.2**. The bags were covered by straw in order to prevent from the dryness and straw was removed when seedling was emerged. For this experiment 11 test crops were planted randomly in 33 polyethylene bags in triplicate for each type of test crops and 3 polyethylene bags were left fallow as a control.

Table 3.2. List of selected winter crops, their number sown / polyethylene bag and number maintained / polyethylene bags, for study of *Orobanche* seed bank in soil.

Test crops	**Seeds**	**Plants**
Botanical name	**sown/bag**	**maintained/bag**
*Linum usitatissimum**	10	3
*Foeniculum vulgare**	10	5
*Solanum tuberosum****	3	1
*Trigonella foenum-graecum**	10	5
*Glycine max**	5	3
*Allium cepa****	3	1
*Capsicum frutescens***	3	2
*Vici faba**	5	3
*Allium sativum****	5	3
*Raphanus sativa**	5	2
*Pisum sativum**	5	3

*Seed, **Seedling, ***Bulb-let, ****Bulb, ****Tuber

3.4 Soil sampling

The pre-sowing and post-harvest soil sampling were collected by composite sampling method along three rows from 9 spots from the depth up to 15 cm and finally half kg of soil from each plot was collected in a polyethylene bag which is well labeled and carried to the laboratory.

3.5.1 Seed standardization

Seed recovery was done from the soil with following method of sieving and differential floatation technique of Ashworth (1976) with some modification (Khattri *et al.,* 1997; Acharya *et al.,* 2003) was used for the *Orobanche*. The standardization of seed was done in four different types of soil namely, sandy, sandy loam, loam and clayey soil.

One hundred *Orobanche* seeds were mixed with 100 g of soil sample in a 250ml beaker. Tap water was added filling upto three fourth of the beaker. This mixture was stirred with glass rod and left for one day. Then the mixture was again stirred and poured into the two sieves (upper sieve size was of 279 micron and lower ones was of 125 micron). The mixture was forced to run down the sieve by full force of shower so that the *Orobanche* seed and small sized soil particles were collected in lower sieve (125 micron). The soil particles were transferred into 100 ml beaker containing calcium chloride (40%w/v) of specific gravity 1.3957, which was collected in lower sieve. It was left undisturbed for some time so that the heavier particles settle down. The floating material was carefully transferred into small sieve of 6-inch diameter of 100 microns. The small sieve containing *Orobanche* seed and soil debris was washed several times with water and treated with 2% sodium hypochlorite for 1-2 minute. The remaining materials (seed and debris) was washed with distilled water for several times and transferred into lined muslin cloth (1-25 lines were drawn on muslin cloth spacing about 1cm). The seeds along with other organic debris were spread evenly with the help of brush or needle on cloth spread over the glass slab and observed under the stereomicroscope (40X magnification). The *Orobanche* seeds were identified from material due to its ornamented seed coat, shape and size. There were altogether 20 samples for four different types of soil, it means that five replications for each types of soil. The total number of recovered seeds in the soil samples was calculated with a correction factor based on the percentage of seeds recovered (Lopez Granados and Garcia Torres, 1993).

3.5.2 *Orobanche* seed estimation

Seed bank of *Orobanche* spp. in infested field in Sarlahi, Lalbandi was studied with above mentioned method. The results obtained were analyzed statistically. The actual numbers of *Orobanche* seeds recovered were calculated by the following formula (Lopez-Granados and Garcia-Torres, 1993).

$$\text{Actual number of } \textit{Orobanche} \text{ seeds} = \frac{\text{No.of recoverd } \textit{Orobanche} \text{ seeds per 100 g soil}}{\textit{Orobanche} \text{ seed standarization index*}} \times 100$$

*mean value of *Orobanche* seed recovered in four different types of soil.

The number of *Orobanche* seeds per meter square was determined by using the following formula of Sauerborn *et al.*, (1991):

No. of *Orobanche* seed/m2 = No. of *Orobanche* seeds/100gm × soil depth (cm) × soil density × 100.

The percentage reduction of *Orobanche* seeds were determined from the difference of initial *Orobanche* seed count before sowing and final seed count after harvest.

3.6 Effect of cropping pattern on emergence of *Orobanche*:

To evaluate the effects of different crops on emergence of *Orobanche*, field history of different fields having different crops in ward no. 8 of Lalbandi was recorded through interview with concerned farmers having different crops. The field history of last 10 years was noted. Along with this, farmers' method to control *Orobanche* emergence also noted. *Orobanche* emergence in the field along with the crops was recorded in 1m×1m quadrat. Five quadrats were laid in each field.

3.7 Statistical analysis

To understand if the seed bank differed significantly with different non-host crops, seed bank data obtained from pre sowing and post- experiments were statistically analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at P=0.05 level of significance in SPSS program Version 21.

CHAPTER 4: RESULTS

4.1 Quantitative estimation of *Orobanche* seeds in infested soil

4.1.1. Standardization of *Orobanche* seed estimation

The recovery percentage of *Orobanche* seeds from different types of soil showed that their recovery varies according to soil types. Highest percentage of *Orobanche* seeds recovery was obtained in sandy soils (69.8±7.26) followed by sandy loam, loam, and clay soil i.e. 64.4±10.74, 64.2±10.89, and 56.8±12.99 respectively (**Figure. 4.1**).

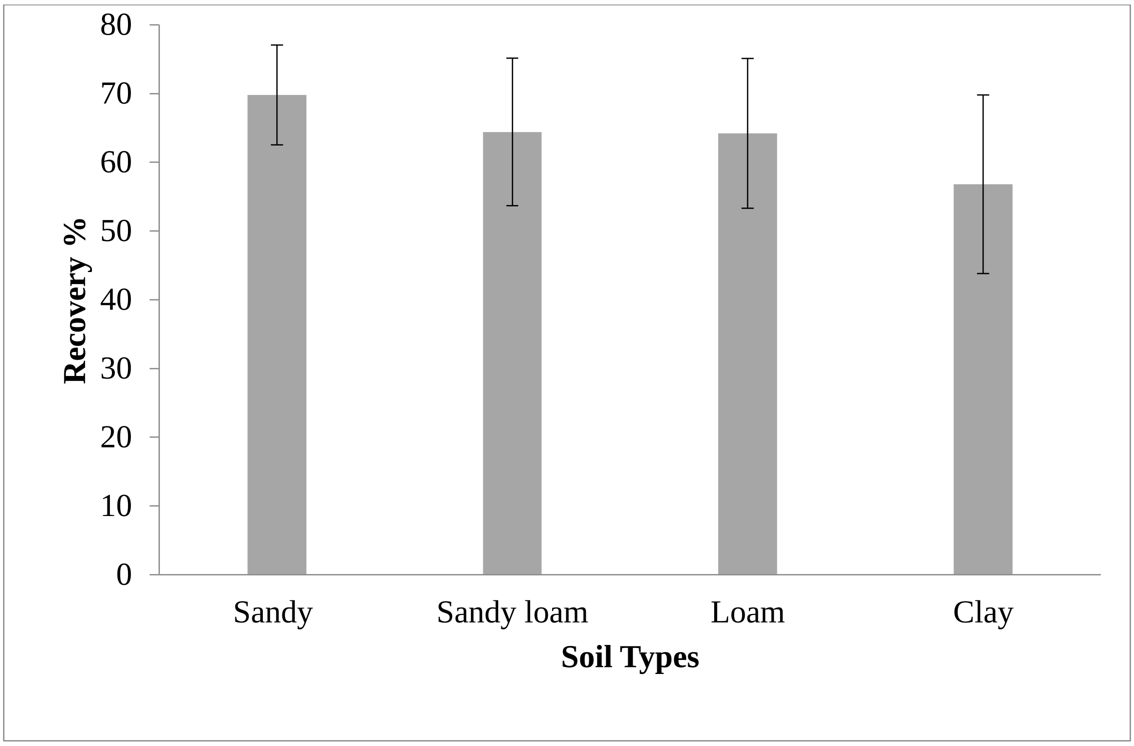

Figure 4.1. Recovery of *Orobanche* seeds from the different type's soil (n=5)

4.2 Effects of different test crops on *Orobanche* seed bank in infested soil

The comparison between the seed bank of pre sowing and post-harvest of test crops showed that the reduction of *Orobanche* seeds occured in all polyethylene bags and field plots including fallow treatment (**Figure 4.2)**.

4.2.1 Impacts of test crops on *Orobanche* seed bank in polyethylene bags

The average number of *Orobanche* seed/100g soil at the time of pre sowing and post-harvest of test crops in polyethylene bags experiment including fallow (as control) is given in (**Figure. 4.2**). The figure showed that there is a reduction of seed in all test crops including fallow. The fallow (36.21±2.62) showed more reduction in *Orobanche* seed than *Raphanus sativus* (22.65±10.52) crops but insignificant.

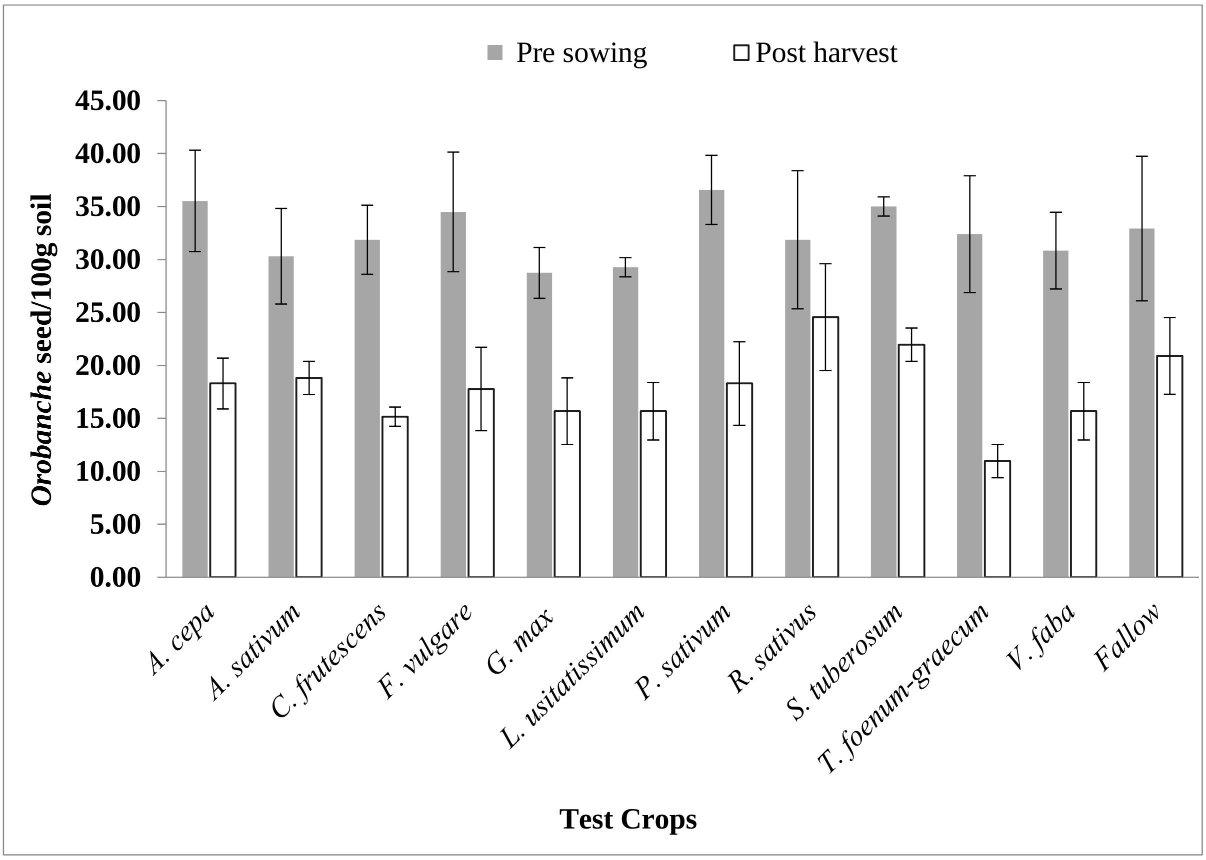

Figure 4.2. Average number of *Orobanche* seed/100g soil of pre-sowing and post-harvest of test crops in Polyethylene bag experiment.

The percentage of *Orobanche* seeds reduction ranges from (22.65±10.52) to (64.87±11.14) (**Table 4.1**). The *Orobanche* seed bank in soil reduced significantly (P=0.05) by fenugreek (*Trigonella foenum-graecum*), chilli (*Capsicum frutescens*), faba bean (*Vicia faba*) and pea (*Pisum sativum*) compared to fallow. The highest *Orobanche* seed reduction was found in *Trigonella foenum-graecum* (64.87±11.14). Insignificant reduction in *Orobanche* seed bank was recorded with test crops potato (*Solanum tuberosum*), onion (*Allium cepa*), garlic (*A. sativum*), soyabean (*Glycine max*) and fennel (*Foeniculum vulgare*), (**Table 4.1**).

Table 4.1. Reduction (%) of *Orobanche* seed density in different test crops grown in pots.

Test Crops	Common Name	% Reduction in Seed Density
		Mean±Sd
Allium cepa L.	Onion	37.40±5.84 BC
Allium sativum L.	Garlic	48.18±7.44 CD
Capsicum frutescens L.	Chilli	52.05±7.05 D
Foeniculum vulgare Mill.	Fennel	47.92±11.50 CD
Glycine max (L.) Merr.	Soybean	45.80±6.58 CD
Linum usitatissimum L.	Linseed	46.58±7.76 CD
Pisum sativum L.	Pea	50.38±6.58 CD
Raphanus sativus L.	Radish	22.65±10.52 A
Solanum tuberosum L.	Potato	37.27±6.19 BC
Trigonella foenum-graecum L.	Fenugreek	64.87±11.14 E
Vicia faba L.	Faba bean	49.39±3.07 CD
Fallow (control)		36.21±2.62 BC

(Same letters followed after the mean ± standard deviation does not differ significantly at P=0.05 according to the Duncan's Multiple Range Test followed after ANOVA)

4.3 Impacts of test crops on *Orobanche* seed bank in field

The number of *Orobanche* seed/sq.meter soil at the time of pre sowing and post-harvest of test crops in field experiment including fallow (as control) is given in **Figure. 4.3.** The result showed that there is a reduction of seed in all test crops including fallow. The crops like radish (*Raphanus sativus*) (28.04±6.32) and soybean (*Glycine max*) (30.48±4.03) showed the insignificant reduction of *Orobanche* seed than fallow (38.16±8.37). The percentage of *Orobanche* seeds reduction ranges from (28.04±6.32) to (70.29±7.85).

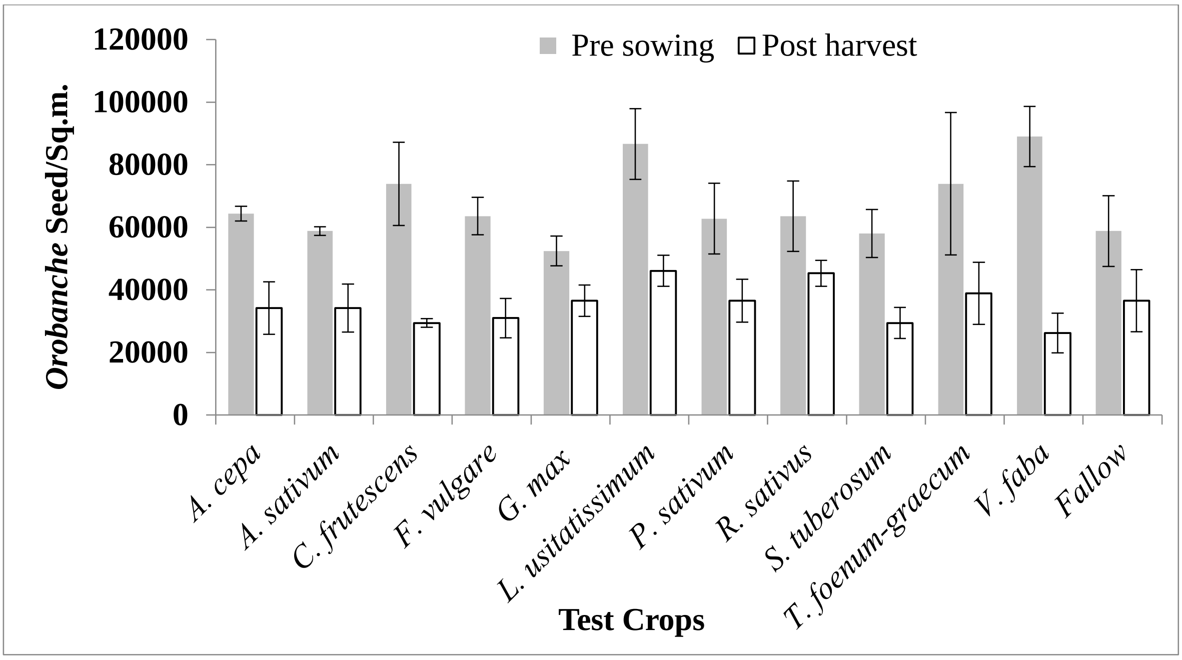

Figure 4.3. Average number of *Orobanche* seed/Sq.m. soil of pre-sowing and post-harvest of different test crops in Field experiment.

The significant reduction of *Orobanche* seed bank in soil was found in faba bean (*Vicia faba*) and chilli (*Capsicum frutescens*) compared to control field experiment. The highest *Orobanche* seed reduction was found in faba bean (*Vicia faba*) (70.29±7.85) **(Table 4.2).**

Table 4.2. Reduction (%) of *Orobanche* seed density in different test crops grown in field

Test Crops	Common Name	% Reduction in Seed Density
		Mean±Sd
Allium cepa L.	Onion	47.18±11.02 BC
Allium sativum L.	Garlic	41.67±14.57 AB
Capsicum frutescens L.	Chilli	59.57±5.52 CD
Foeniculum vulgare Mill.	Fennel	51.59±5.84 BC
Glycine max (L.) Merr.	Soybean	30.48±4.03 A
Linum usitatissimum L.	Linseed	45.84±12.05 BC
Pisum sativum L.	Pea	41.83±2.21 AB
Raphanus sativus L.	Radish	28.04±6.32 A
Solanum tuberosum L.	Potato	49.08±8.63 BC
Trigonella foenum-graecum L.	Fenugreek	46.67±5.77 BC
Vicia faba L.	Faba bean	70.29±7.85 D
Fallow (control)		38.16±8.37 AB

(Same letters followed after the mean ± standard deviation does not differ significantly at P=0.05 according to the Duncan's Multiple Range Test followed after ANOVA)

4.4 People's perception

The local people of Lalbandi were aware about the *Orobanche* spp. and its effects. Different control methods such as spraying liquid mobile on stem apex of the *Orobanche* shoot, hand weeding, herbicide spray, crop rotation and intercropping were found to be practiced, but none of the methods were effective. Many farmers were discouraged for tomato farming due to frequent *Orobanche* invasion. Due to its invasion some farmers have reported to have shifted from tomato fields to Mango, litchi orchards or *Eucaluptus* plantation. Some farmers have started crop rotation like maize- tomato-brinjal, cucumber-tomato-brinjal, chick pea-maize, lablab bean –maize-soya bean. Despite of *Orobanche* infestations, most of the local people still cultivate the host plants (brinjal and tomato) because these crops are cash crops, which earn more profit than other crops and also rely on this regular source of income.

4.5 Farmers' Field observation

Ten different fields having different field history were studied for *Orobanche* emergence which is given in **Table 4.3**.

Table 4.3. Field observation and *Orobanche* emergence

S.N.	Crops Field	Field History
1	Tomato field A	Paddy cultivation in practice last 10 years in rainy season and this year in rainy season cucumber was cultivated instead of Paddy. Emergence of very few *Orobanche* shoots was observed in such tomato cultivated field. Paddy-Cucumber-Tomato
2	Tomato field B	Maize-tomato crop rotation. Quite high *Orobanche* emergence. Maize- Tomato
3	Tomato field C	Last year cowpea was cultivated, followed by maize and now tomato. High *Orobanche* emergence. Cowpea-Maize-Tomato.
4	Tomato field D	Maize was continuously cultivated for 3-year and then last one year ago during winter season lablab bean (*Dolichos lablab)*. Lablab bean was grown, in following season (rainy season) brinjal was cultivated. And now tomato was planted. No *Orobanche* emergence. Maize-lablab bean -Brinjal-Tomato

5	Brinjal field A	2 years earlier brinjal was cultivated. After that inter cropping of lentil and chickpea and now brinjal, very few emergences of *Orobanche* spp. Brinjal - lentil and chickpea- maize - brinjal.
6	Brinjal field B	Previous crop was pointed gourd and then cucumber and now brinjal. High *Orobanche* emergence Pointed Gourd- Cucumber-Brinjal
7	Brinjal field C	In two successive season (winter and summer) maize was planted and in winter brinjal. Very high *Orobanche* emergence. Maize- maize-Brinjal
8	Brinjal field D	Maize was cultivated in winter followed by cucumber in summer and then brinjal. The host plant (brinjal) were wilted and died, which may be due to other pathogens or sever impact of *Orobanche* (but very few *Orobanche* shoot was observed). Farmers use the vacant space after removing dead brinjal plants to grow tori and that appears as intercropping. A few *Orobanche* emergence. Maize- Cucumber- brinjal
9	Mustard field	Crops rotation with paddy and mustard for last 10 years. High emergence of *Orobanche* Paddy-Mustard
10	Mixed cropping (Chickpea and Mustard) field	Maize in summer and mixed cropping of Chickpea-Mustard in winter. A few *Orobanche* emergence Maize in summer-(chick pea-mustard) in winter

Out of 10 fields, four fields were cultivated with the host crop tomato, four fields with brinjal, one with mustard and one field with mixed crops of chickpea and mustard. *Orobanche* emergence in these fields were recorded in $1\times1m^2$ plots and the results are given in **Table 4.4.** Highest emergence of *Orobanche* shoot was observed in the brinjal field C. (24.8±23.88), which was with brinjal-maize crop rotation. In tomato field D, no emergence of *Orobanche* shoot was observed. In the tomato field A and field with mixed cropping (chickpea and mustard), less emergence of *Orobanche* shoot (0.6±0.55 and 1.6±0.89) was observed.

Table 4.4. Types of field, mean number of host and *Orobanche* with their respective S.d.

S.N.	**Types of Field**	**Mean no. of Host and Sd/m²**	**Mean no. of *Orobranche* and Sd/m²**
1	Tomato field A	4.8±0.84	0.6±0.55
2	Tomato field B	4.4±0.55	10.6±5.41
3	Tomato field C	3.2±0.84	6±4.69
4	Tomato field D	3±0.71	0±0
5	Brinjal field A	1.8±0.84	0.4±0.20
6	Brinjal field B	3.4±0.55	6.4±5.89
7	Brinjal field C	2±0.71	24.8±23.88
8	Brinjal field D	1.4±0.55	2.4±1.14
9	Mustard field	38.6±13.01	6.6±5.32
10	Chickpea and Mustard field	12.6±4.16	1.6±0.89

CHAPTER 5: DISCUSSIONS

5.1 *Orobanche* seed estimation

The study of importance of seed bank in infested soil has been emphasized by many workers for the proper management of weed (Sauerborn *et al.,* 1991, Auld *et al.*, 1979). The study of soil seed bank is found to be more reliable method of infestation than other visuals field surveys (Sauerborn *et al.*, 1991)

Different workers have used different method for the estimation of *Orobanche* seeds in infested soil. In the method developed by the working group at ICARDA (International center for Agriculture Research in the Dry Areas, Aleppo, Syria) $MgSO_4$ solution of specific gravity of 1.16 g/cm^3 was used for the suspension of *Orobanche* seeds (Kachelriess, 1987). Ashworth (1976) used 40% (w/v) $CaCl_2$ solution of specific gravity 1.3957 for quantitative detection of *Orobanche* seeds in infested soil.

The technique used in the present study modified principally follows the sieving and flotation technique of Ashworth, (1976) with some modification (Acharya *et al.,* 2003) to isolate the *Orobanche* seeds from heavier organic particles. The recovery percentage in the present study resembles with that of Acharya, (2002). The high recovery in sandy soil is mainly due to presence of large soil particles (0.02 to 2mm diameter) and less organic matter. As the size of soil particles in sandy soil is large, most of them were retained in upper sieve (of 279 micron) and makes the seed counting easier. In sandy loam, loam and clay soil, there were presence of more organic matter and soil debris with nearly similar size to *Orobanche* seeds, which not only created error in the seed recovery but also made seed counting difficult.

The seed not recovered may lost during floatation in present method. The loss of some *Orobanche* seed during centrifuge had also mentioned by Ashworth (1976).

5.2 Investigation of trap crops

Different crops have different ability to induce the germination of *Orobanche* seed. This is mostly due to presence of root exudates, which stimulate seed germination (Abbes *et al.,* 2008). *Orobanche* seed bank in soil was estimated before sowing and after harvest of different crop in polyethylene bags and fields. In most of the cases the seed bank reduction was found to be higher in polyethylene bag experiment than in field experiments, this may be due to the higher concentration of roots, and controlled environment. The findings of *Orobanche* seed bank reduction in soil after sowing of different test crops have been discussed below in each test crops investigated:

5.2.1 Control polyethylene bags/plots (Fallow)

In the present study, it was interesting to note down that there was some reduction in *Orobanche* seed bank in fallow polyethylene bags and field plots. This might be due to the fact that weed seed density is not only affected by host stimulant but also by environmental factors like rainfall, wind tillage practices, kind of manure used, activities of soil animals and soil microorganism (Lopez Granados and Garcia Torres 1993). In the present study, mass of isolated *Orobanche* seeds from infested soil samples contained a fair number of damaged seeds. Some of the damaged seeds were found infested with fungus, some empty (without embryo) and some broken possibly devoured by soil insects (Acharya, 2002).

5.2.2 *Allium cepa* L. (Onion)

The reduction of *Orobanche* seed bank with plantation of onion were found to be insignificant in both experiments (field and polyethylene bag) whereas some author reported that this crop as moderately potential trap crop for *Orobanche aegyptiaca* (Acharya, 2012) some author observed *Allium cepa* parasitized by *Orobanche cernua* (Akhter *et al.*, 2018). So, further investigation is needed effects of *Orobanche* spp. on onion. But in our study, there was no such attachment found on onion and insignificant *Orobanche* seed reduction. Thus from our experimental data, onion can be used as non-potential trap crop.

5.2.3 *Allium sativum* L. (Garlic)

In the present study, the reduction of *Orobanche* seed bank with plantation of garlic were found to be insignificant in both field and polyethylene bag experiments than in fallow. Though it was described as trap crop for *O. aegyptiaca* by Abu-Irmaileh (1984), in present study it cannot reduce the *Orobanche* seed significantly which is supported by Acharya (2012). Thus from present experimental data, garlic cannot be used as non-potential trap crop.

5.2.4 *Capsicum frutescens* L. (Chilli)

Bischof and Koch (1974) reported pepper as trap crop for Egyptian broomrape. Hershenhorn *et al.*, (1996) observed that more than 50% of Egyptian broomrape seeds germinated in the presence of all pepper variety roots except the sweet pepper. However only a few germinated seeds attached with the host and developed. The two paprika varieties Lehava and Shani were hosts to more parasites than the sweet pepper varieties, and showed as many as 15 parasites per host plants. Pepper roots stimulated germination of 22-26% of nodding broomrape (*Orobanche cernua*) seeds without any parasitic attachment (Hershenhorn et al., 1996). Jaconbshon et al., (1991) also reported that root exudates of pepper stimulate the seed germination of broomrapes without any infection.

In the present study some of the *Orobanche* shoots were found to be attached with the roots of *Capsicum* in the fields. Besides this significant reduction of *Orobanche* seed in both field and polyethylene experiments indicated that *Capsicum frutescens* has ability to stimulate *Orobanche* seed germination and also has probability for attachment, which resembles with the observation of Hershenhorn *et al.,* (1996) and Jaconbshon *et al.,* (1991). Hence, *Capsicum frutescens* can be used as potential trap crop.

5.2.5 *Foeniculum vulgare* Mill. (Fennel)

It is regarded as host crop of *Orobanche ramosa*, *O. aegyptiaca* and *O. crenata* and crop damages have been reported by Foy *et al.,* (1989) and Parker (1986). Khattri (1997) described it as an occasional host of *O. aegyptiaca* but Acharya (2012) described fennel as trap crop for the integrated management of *O. aegyptiaca* in infested (*Brassica campestris* var. Tori) field.

In present study fennel showed insignificant seed reduction of *Orobanche* spp. in both polyethylene bags and field experiment. So it cannot be used as possible trap crops but it can be used in infested tomato field in crop rotation, it is used as green vegetation and also as spices.

5.2.6 *Trigonella foenum-graecum* L. (Fenugreek)

Al-Menoufi (1991); Bakheit *et al.,* (2002), reported it as trap crop that helps to reduce the infection of *Orobanche crenata* when fenugreek and faba bean intercroped. Abbes *et al.* (2008) also recognized fenugreek as trap crops after observing *O. crenata* seed germination on their roots in vitro. Our result of *Orobanche* seed bank after using fenugreek in both polyethylene bag and field experiments support the above observations. High seed bank reduction in the polyethylene bag may be due to high root exudates concentration than in field condition. Thus, fenugreek can be used as potential trap crops according to this experimental study.

5.2.7 *Linum usitatissimum* L. (Linseed)

Linseed is the crops where more research has been done for the control of parasitic plants such as *Orobanche* spp. So some author reported as trap crops for *Orobanche crenata* (Ibrahim *et al.*, 1973; Arslan and Uygur, 2013) *O. cernua* Loefl. (Krishnamurty *et al.*, 1977) and *O. ramosa*

(Hameed *et al.,* 1973). According to the Babaei *et al.*, (2010) linseed can reduced the broomrape biomass by 75.2% and consequently increases the tomato yield. Kleifeld *et al.,* (1994), observed that growing linseed in pot in two successive winter seasons or one summer cropping with mungbean reduced the early infestation of *Orobanche aegyptiaca* and significantly increased tomato vigor and production. In contrast *O. aegyptiaca* attack parasitised the linseed and developed inflorescences. Various sources of the parasite showed different degrees of virulence to linseed, from heavy attack and severe damage to sparse attachment with no production of flowering shoot. The virulent species attacked linseed all year round, causing severe damaged. Similar result was also observed by Goldwasser *et al.,* (1991) and reported that linseed was heavily parasitized by *O. aegyptiaca* as shown in figure and therefore, it was considered as catch crops in those studies and similar result has been reported from this research work that Linseed was parasitized by *Orobanche* as shown in **Annex 3 (photo plate S)**.

Acharya, (2012) reported linseed as a potential trap crops for the *Orobanche aegyptiaca* in infested tori field. In the present study slight reduction in *Orobanche* seed bank in both field and polyethylene bag experiments indicated it to be moderate trap crops.

5.2.8 *Vicia faba* L. (Faba bean)

Schnell *et al.,* (1994) demonstrated that the faba bean was most effective for the *Orobanche crenata* which reduced the seed bank by (47.3%) and fallow (10.6%) the least effective. The faba bean root exudates revealed clear gibberellin activity, which is responsible for the stimulation of *Orobanche crenata* seeds. Aalders and Pieters (1987) observed a positive correlation between the biomass of *Orobanche crenata* and the biomass of faba bean. It has been reported as a rare host of *O. aegyptiacai* in Nepal (Khattri, 1997).

So above result supports the findings of present study of the field experiment which showed significant seed reduction whereas in polyethylene bags showed insignificant reduction. Thus, it can be used as potential trap crops in infested tomato field.

5.2.9 *Glycine max* (L.) Merr. (Soyabean)

Soyabean is a leguminous plant that have allelopathic effects on other plants. Many studies revealed that it inhibited the germination and growth of speargrass (*Imperata cylindrical* L.) (Olubunmi, *et al.,* 2012). Similar result also supported by Zhang *et al.,* (2013) that methanolic extracts of soybean roots induced higher germination of broomrape than stem and leaf extracts. The broomrape germination rates induced by root extracts were also positively correlated with diameter of soybean nodule and dry weight. The results indicated that soybeans could induce sunflower broomrape germination and can be used as potential trap crop. Intercropping soybean with maize can reduce the parasitism rate of *Striga hermonthica* (Del.) Benth, resulting in increased maize yield (Odhiambo, *et al.,* 2011). In present study seed reduction was slightly insignificant in polyethylene bags experiment but was totally insignificant in field experiments. Hence the present study does not support the above result. However, it can be cultivated in summer for crop rotation, which will increase the nitrogen content of soil and indirectly or directly may reduce *Orobanche* seed germination (Bista, 2014 and Habimana *et al*., 2014).

5.2.10 *Raphanus sativus* L. (Radish)

Acharya, (2012) also considered it as a potential trap crops to decreased the *Orobanche aegyptiaca* seed of highly infested of *Brassica campestris*. Cabbage (*Brassica oleracea* L.) of the same family brassicaceae, reduced the number of broomrape (*Orobanche ramosa* L. / *O. aegyptiaca* Pers.) branches by 28% in potato fields in Turkey (Nemli *et al.,* 2009). In contrast, in the present study insignificant seed reduction of *O. cernua* in both field and polyethylene bags experiment was observed at Lalbandi in tomato fields. So, from this experiment it can be considered as a non-potential non-host crop.

5.2.11 *Solanum tuberosum* L. (Potato)

Saurborn, (1991a); Foy *et al.,* (1989) has described potato as a very common host of *Orobanche*. *aegyptiaca* and *O*. *muteli*, which causes severe crop damage. But in Nepal, there is no report regarding potato being infected by any of the *Orobanche* spp. Occurring in Nepal (Khattri, 1997).

In present study, the number of *Orobanche* seeds in infested soil in polyethylene bags and field experiments after potato cultivation reduced insignificantly compared to control treatments. No attachment or emergence of *Orobanche* was seen in the experimental study during the time of harvesting. So, it cannot be used as effective trap crops, but its cultivation is possible in *Orobanche* infected tomato field.

5.2.12 *Pisum sativum* L. (Pea)

About 500,000 ha. of pea cultivated areas in Mediterranean and West Asia have been reported to be infested and endangered by *Orobanche crenata* (Sauerborn, 1991b). Fortunately, the infestation of *Orobanche* species on pea or other legume crops is not common in Nepal. In present study, reduction of *Orobanche* seed was observed in polyethylene bags experiment compared to control treatments but insignificant result was observed in field experiment. So, it cannot be used as effective trap crops.

5.3 Farmers' Field observation:

No emergence of *Orobanche* was observed in Tomato Field D, which might be either due to repeated hand pulling as the field size was smaller than the others or might be due to plantation of lablab bean, but needs further investigation. Very few *Orobanche* emergence in Brinjal Field A, might be due to intercropping of lentil and chickpea and maize cultivation. This observation of Brinjal Field A *Orobanche* emergence further supports seed bank study conducted in Lalbandhi (Jyawali, 2020). Similarly, a few emergences in Brinjal Fied D was observed, which might be due to plantation of cucumber in summer, but needs further investigation. Fields with intercropping of chick pea + mustard in winter and maize in summer also showed very less emergence of *Orobanche*, which might be due to the potentiality of chick pea and this observation further supports the finding of seed bank study of Jyawali (2020) at Lalbandhi.

CHAPTER 6: CONCLUSION AND RECCOMENDATION

6.1 Conclusion

Quantitative evaluation of *Orobanche* and *Phelipanche* spp. seeds from the infested soil has been one of the major problems of *Orobanche* and *Phelipanche* spp. Efforts have been developed to make this method reliable, easier and faster. In the present study a method given by Ashworth (1976) with some modification given by Acharya *et al.* (2003) was used to make it less expensive. And this method was found to be fairly simple, easier and reliable. The present study of *Orobanche* seed estimation technique for the recovery of seed not only depended on soil texture but also on amount of organic matter present.

From the present experimental study for potential trap crops in polyethylene bag and field clearly showed that crops like faba bean (*Vicia faba*) and chilli (*Capsicum frutescense)* ranked highest among the crops which reduce *Orobanche* seed bank significantly.

On the basis of results obtained from present study in both polyethylene bag and field, the crops investigated could be listed in the following categories:

Highly potential trap crops: *Vicia faba* and *Capsicum frutescens.*

Moderately potential trap crops: *Trigonella foenum-graecum*, *Foeniculum vulgare, Linum usitatissimum, Glycine max* and *Pisum sativum*.
Non-potential trap crop: *Allium sativum*, *Allium cepa*, *Raphanus sativus*, and *Solanum tuberosum*.

Finally, from this investigation it can be concluded that any of the crops listed in the category of highly potential trap crops (*Vicia faba* and *Capsicum frutescens*) could be used in crop rotation along with the hand weeding, intercropping, catch crops which is an important component of integrated management of *Orobanche* and *Phelipanche* spp. in highly infested area.

6.2 Recommendation

From the present study it can be recommended to farmers that any of the crops listed in the category of highly potential trap crops (*Vicia faba* and *Capsicum frutescens*) could be used in crop rotation along with the integrated management methods to reduce the seed bank of *Orobanche* in infested tomato field of Lalbandhi, Sarlahi.

The farmers are also recommended to collect the *Orobanche* diseased crops plants and the shoots of *Orobanche* and burned down them in ordered to prevent them adding infestation level and spreading. From the farmer's field observation, rotation of crops with chick pea, maize and lentil are recommended to cultivate to reduce *Orobanche* seed bank.

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Annexes

Annex 1: Field preparation and plantation of test crops.

Photo plate A : Field preparartion.

Photo plate B:Plantation of chilli seedling

Photo plate C: Germination of test crops in field plot.

Photo plate D:Germination of test crops in Polyethylene bag.

Photo plate E: Weeding and thinning of test crops.

Photo plate F: Watering of test crops.

Annex 2: Hand weeding of test crops on field and polyethylene bags

	
Photo plate G: Hand weeding on onion	Photo plate H: Hand weeding on soybean
	
Photo plate I: Different test crops in polyethylene bags	Photo plate J: Different test crops in polyethylene bags
	
Photo plate K: Harvesting radish from polyethylene bag.	Photo plate L: Matured pea plant on polyethylene bag.

Annex 3: Flowering and fruiting stages of different test crops.

	
Photo plate M: Matured stage of chilli	Photo plate N: Fennel flowering stage
	
Photo plate O: Faba bean flowering and fruiting stage	Photo plate R: Linseed flowering and fruiting stage

Photo plate S: Emergence of *Orobanche* on Linseed